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**United States Patent** [19][11] **Patent Number:** **6,096,504****McGonigle et al.**[45] **Date of Patent:** **Aug. 1, 2000**[54] **MAIZE GLUTATHIONE-S-TRANSFERASE ENZYMES**[75] Inventors: **Brian McGonigle**, Wilmington, Del.;  
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Wilmington, Del.[21] Appl. No.: **09/248,335**[22] Filed: **Feb. 10, 1999****Related U.S. Application Data**[63] Continuation-in-part of application No. 08/924,759, Sep. 5,  
1997, Pat. No. 5,962,229.[51] **Int. Cl.**<sup>7</sup> ..... **C12Q 1/68**; C12N 1/20;  
C12N 5/00; C07H 21/04[52] **U.S. Cl.** ..... **435/6**; 435/193; 435/455;  
435/410; 435/252.33; 435/320.1; 536/23.1;  
536/23.2; 536/23.6[58] **Field of Search** ..... 435/193, 252.33,  
435/410, 320.1, 6, 455; 536/23.2, 23.1,  
23.6[56] **References Cited****U.S. PATENT DOCUMENTS**5,073,677 12/1991 Helmer et al. .... 800/205  
5,589,614 12/1996 Bridges et al. .... 800/205**FOREIGN PATENT DOCUMENTS**0 256 223 5/1987 European Pat. Off. .  
WO 93 01294 1/1993 WIPO .  
WO 96/23072 8/1996 WIPO .  
WO 97/11189 3/1997 WIPO .  
WO99/14337 9/1999 WIPO .**OTHER PUBLICATIONS**David C. Holt et al., Characterization of the Safener-Induced Glutathione S-Transferase Isoform II from Maize, *Planta*, 196, 295-302, 1995.F. Droog, Plant Glutathione S-Transferases, a Tale of Theta and Tau, *J. Plant Growth Regul.*, 16, 95-107, 1997.Laura Rossini et al., Characterization of Glutathione S-Transferase Isoforms in Three Maize Inbred Lines Exhibiting Differential Sensitivity to Alachlor, *Plant Physiol.*, 112, 1595-1600, 1996.Kathleen A. Marrs, The Functions and Regulation of Glutathione S-Transferases in Plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 47, 127-158, 1996.Sharad S. Singhal et al., Purification and Characterization of Glutathione S-Transferase from Sugarcane Leaves, *Phytochemistry*, 30, No. 5, 1409-1414, 1991.Robert Edwards et al., Glutathione Transferases in Wheat (Triticum) Species with Activity toward Fenoxaprop-Ethyl and Other Herbicides, *Pesticide Biochemistry and Physiology*, 54, 94-104, 1996.Michael A. Wosnick et al., Total Chemical Synthesis and Expression in *Escherichia coli* of a Maize Glutathione-Transferase (GST) Gene, *Gene*, 76, 153-160, 1989.Ian Jepson et al., Cloning and Characterization of Maize Herbicide Safener-induced cDNAs Encoding Subunits of Glutathione S-Transferase Isoforms I, II, and IV, *Plant Molecular Biology*, 26, 1855-1866, 1994.Dianne A.M. van der Kop et al., Isolation and Characterization of an Auxin-Inducible Glutathione S-Transferase Gene of *Arabidopsis Thaliana*, *Plant Molecular Biology*, 30, 839-844, 1996.Czarnecka et al. *Mol. Cell. Biol.*, 8(3), 1113-1122, 1988.Dilip M. Shah et al., Structural Analysis of a Maize Gene Coding for Glutathione-S-Transferase Involved in Herbicide Detoxification, *Plant Molecular Biology*, 6, 203-211, 1986.Robert E. Moore et al., Cloning and Expression of a cDNA Encoding a Maize Glutathione-S-Transferase in *E. Coli*, *Nucleic Acids Research*, 14, No. 18, 7227-7235, 1986.Kriton K. Hatzios et al., Herbicide Safeners, *J. Environ. Sci. Health*, B31(3), 545-553, 1996.Thomas Flury et al., A 2,4-D-Inducible Glutathione S-Transferase from Soybean (Glycine Max), *Physiologia Plantarum*, 94, 312-318, 1995.Robert Edwards, Characterization of Glutathione Transferases and Glutathione Peroxidases in Pea, *Physiologia Plantarum*, 98, 594-604, 1996.McGonigle, Brian et al., Hemoglutathione selectivity by soybean, *Pestic. Biochem. Physiol.* (1998), 62(1), 15-25.Koeppel et al., Role of glutathione conjugation in the detoxification of sulfonylurea herbicides in plants, Book of Abstracts, 216<sup>th</sup> American Chemical Society, (1998), (Abstract).Grove et al., Characterization and Heterospecific Expression of cDNA Clones of Genes in the Maize GSH S-Transferase Multigene Family, *Nucleic Acids Research*, vol. 16, No. 2, 425-438, Jan. 1, 1988.Dixon et al., Purification regulation and cloning of a glutathione transferase (GST) from maize resembling the auxin-inducible type-III GST's *Plant Molecular Biology*, vol. 36, 75-87, Jan., 1998.

(List continued on next page.)

*Primary Examiner*—Nashaat Nashed[57] **ABSTRACT**

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of maize glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of maize GST enzymes, host cells transformed with those genes and methods of the recombinant production of maize GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying maize GST enzyme substrates and maize GST enzyme inhibitor, are also provided.

**8 Claims, No Drawings**

OTHER PUBLICATIONS

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Dixon et al., Glutathione-mediated detoxification systems in plants, *Current Opinion in Plant Biology*, vol. 1, No. 3, Jun. 1998, pp. 258-266.

Timmerman, Molecular Characterization of Corn Glutathione S-Transferase isozymes involved in Herbicide Detoxification, *Physiologia Plantarum*, vol. 77, No. Symp.01, Jan. 1, 1989, pp. 465-471.

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## MAIZE GLUTATHIONE-S-TRANSFERASE ENZYMES

This is a continuation-in-part of application Ser. No. 08/924,759 filed Sep. 5, 1997, now U.S. Pat. No. 5,962,229. 5

### FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding maize glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. 10

### BACKGROUND OF THE INVENTION

Glutathione-S-transferases (GST) are a family of enzymes which catalyze the conjugation of glutathione, homoglutathione (hGSH) and other glutathione-like analogs via a sulfhydryl group, to a large range of hydrophobic, electrophilic compounds. The conjugation can result in detoxification of these compounds. GST enzymes have been identified in a range of plants including maize (Wosnick et al., *Gene* (Amst) 76 (1) (1989) 153–160; Rossini et al., *Plant Physiology* (Rockville) 112 (4) (1996) 1595–1600; Holt et al., *Planta* (Heidelberg) 196 (2) (1995) 295–302), wheat (Edwards et al., *Pestic. Biochem Physiol.* (1996) 54(2), 96–104), sorghum (Hatzios et al., *J. Environ. Sci. Health, Part B* (1996), B31(3), 545–553), arabidopsis (Van Der Kop et al., *Plant Molecular Biology* 30 (4) (1996), sugarcane (Singhal et al., *Phytochemistry* (OXF) 30 (5) (1991) 1409–1414), soybean (Flury et al., *Physiologia Plantarum* 94 (1995) 594–604) and peas (Edwards R., *Physiologia Plantarum* 98 (3) (1996) 594–604). GST's can comprise a significant portion of total plant protein, for example attaining from 1 to 2% of the total soluble protein in etiolated maize seedlings (Timmermann, *Physiol Plant.* (1989) 77(3), 465–71). 20

Glutathione S-transferases (GSTs; EC 2.5.1.18) catalyze the nucleophilic attack of the thiol group of GSH to various electrophilic substrates. Their functions and regulation in plants has been recently reviewed (Marrs et al., *Annu Rev Plant Physiol Plant Mol Biol* 47:127–58 (1996); Droog, F. *J Plant Growth Regul* 16:95–107, (1997)). They are present at every stage of plant development from early embryogenesis to senescence and in every tissue class examined. The agents that have been shown to cause an increase in GST levels have the potential to cause oxidative destruction in plants, suggesting a role for GSTs in the protection from oxidative damage. In addition to their role in the protection from oxidative damage, GSTs have the ability to nonenzymatically bind certain small molecules, such as auxin (Zettl et al., *PNAS* 91:689–693, (1994)) and perhaps regulate their bioavailability. Furthermore the addition of GSH to a molecule serves as an “address” to send that molecule to the plant vacuole (Marrs et al., *Nature* 375:397–400, (1995)). 25

GSTs have also been implicated in the detoxification of certain herbicides. Maize GSTs have been well characterized in relation to herbicide metabolism. Three genes from maize have been cloned: GST 29 (Shah et al., *Plant Mol Biol* 6, 203–211(1986)), GST 27 (Jepson et al., *Plant Mol Biol* 26:1855–1866, (1994)), GST 26 (Moore et al., *Nucleic Acids Res* 14:7227–7235 (1986)). These gene products form four GST isoforms: GST I (a homodimer of GST 29), GST II (a heterodimer of GST 29 and GST 27), GST III (a homodimer of GST 26), and GST IV (a homodimer of GST 27). GST 27 is highly inducible by safener compounds (Jepson (1994) supra; Holt et al., *Planta* 196:295–302, (1995)) and over- 30

expression of GST 27 in tobacco confers alachlor resistance to transgenic tobacco (Jepson, personal communication). Additionally, Bridges et al. (U.S. Pat. No. 5,589,614) disclose the sequence of a maize derived GST isoform II promoter useful for the expression of foreign genes in maize and wheat. In soybean, herbicide compounds conjugated to hGSH have been detected and correlated with herbicide selectivity (Frear et al., *Physiol* 20: 299–310 (1983); Brown et al., *Pest Biochem Physiol* 29:112–120, (1987)). This implies that hGSH conjugation is an important determinant in soybean herbicide selectivity although this hypothesis has not been characterized on a molecular level.

Some efforts have been made to alter plant phenotypes by the expression of either plant or mammalian foreign GST genes or their promoters in mature plant tissue. For example, Helmer et al. (U.S. Pat No. 5,073,677) teach the expression of a rat GST gene in tobacco under the control of a strong plant promoter. Similarly, Jepson et al. (WO 97/11189) disclose a chemically inducible maize GST promoter useful for the expression of foreign proteins in plants; Chilton et al. (EP 256223) discuss the construction of herbicide tolerant plants expressing a foreign plant GST gene; and Bieseler et al. (WO 96/23072) teach DNA encoding GSTIIIc, its recombinant production and transgenic plants containing the DNA having a herbicide-tolerant phenotype. 15

Manipulation of nucleic acid fragments encoding soybean GST to use in screening in assays, the creation of herbicide-tolerant transgenic plants, and altered production of GST enzymes depend on the heretofore unrealized isolation of nucleic acid fragments that encode all or a substantial portion of a soybean GST enzyme. 20

### SUMMARY OF THE INVENTION

The present invention provides nucleic acid fragments isolated from maize encoding all or a substantial portion of a GST enzyme. The isolated nucleic acid fragment is selected from the group consisting of (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO: 14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72 and SEQ ID NO:74; (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72 and SEQ ID NO:74.; and (c) an isolated nucleic acid fragment that is complementary to (a) or (b). The nucleic acid fragments and corresponding polypeptides are contained in the accompanying Sequence Listing and described in the Brief Description of the Invention. 35



In another embodiment, the instant invention relates to chimeric genes encoding maize GST enzymes or to chimeric genes that comprise nucleic acid fragments as described above, the chimeric genes operably linked to suitable regulatory sequences, wherein expression of the chimeric genes results in altered levels of the encoded enzymes in transformed host cells.

The present invention further provides a transformed host cell comprising the above described chimeric gene. The transformed host cells can be of eukaryotic or prokaryotic origin. The invention also includes transformed plants that arise from transformed host cells of higher plants, and from seeds derived from such transformed plants, and subsequent progeny.

Additionally, the invention provides methods of altering the level of expression of a maize GST enzyme in a host cell comprising the steps of; (i) transforming a host cell with the above described chimeric gene and; (ii) growing the transformed host cell produced in step (i) under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of a plant GST enzyme in the transformed host cell relative to expression levels of an untransformed host cell.

In an alternate embodiment, the present invention provides methods of obtaining a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a maize GST enzyme comprising either hybridization or primer-directed amplification methods known in the art and using the above described nucleic acid fragment. A primer-amplification-based method uses SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 or 73. The product of these methods is also part of the invention.

Another embodiment of the invention includes a method for identifying a compound that inhibits the activity of a maize GST enzyme encoded by the nucleic acid fragment and substantially similar and complementary nucleic acid fragments of SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73. The method has the steps: (a) transforming a host cell with the above described chimeric gene; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a chemical compound of interest; and (e) identifying the chemical compound of interest that reduces the activity of the maize GST enzyme relative to the activity of the maize GST enzyme in the absence of the chemical compound of interest.

This method may further include conducting step (d) in the presence of at least one electrophilic substrate and at least one thiol donor. The isolated nucleic acid fragments of this method are chosen from the group represented by SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73 and the maize GST enzyme is selected from the group consisting of SEQ ID NOS.:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.

The invention further provides a method for identifying a chemical compound that inhibits the activity of the maize

GST enzyme as described herein, wherein the identification is based on a comparison of the phenotype of a plant transformed with the above described chimeric gene contacted with the inhibitor candidate with the phenotype of a transformed plant that is not contacted with the inhibitor candidate. The isolated nucleic acid fragment of this method is selected from the group consisting of SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73 and the maize GST enzyme is selected from the group consisting of SEQ ID NOS.:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.

In another embodiment, the invention provides a method for identifying a substrate for the maize GST enzyme. The method comprises the steps of: (a) transforming a host cell with a chimeric gene comprising the nucleic acid fragment as described herein, the chimeric gene encoding a maize GST enzyme operably linked to at least one suitable regulatory sequence; (b) growing the transformed host cell of step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a substrate candidate; and (e) comparing the activity of maize GST enzyme with the activity of maize GST enzyme that has been contacted with the substrate candidate and selecting substrate candidates that increase the activity of the maize GST enzyme relative to the activity of maize GST enzyme in the absence of the substrate candidate. More preferably, step (d) of this method is carried out in the presence of at least one thiol donor. The isolated nucleic acid fragment of this method is selected from the group consisting of SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73 and the maize GST enzyme is selected from the group consisting of SEQ ID NOS.:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.

Alternatively, methods are provided for identifying a maize GST substrate candidate wherein the identification of the substrate candidate is based on a comparison of the phenotype of a host cell transformed with a chimeric gene expressing a maize GST enzyme and contacted with a substrate candidate with the phenotype of a similarly transformed host cell grown without contact with a substrate candidate.

The isolated nucleic acid fragment of this method is selected from the group consisting of SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73 and the maize GST enzyme is selected from the group consisting of SEQ ID NOS.:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.

#### BRIEF DESCRIPTION OF SEQUENCE DESCRIPTIONS AND BIOLOGICAL DEPOSITS

The invention can be more fully understood from the following detailed description and the accompanying sequence descriptions and biological deposits which form a part of this application.

The following sequence descriptions and sequences listings attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent



applications as set forth in 37 C.F.R. §1.821–1.825. The Sequence Descriptions contain the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IYUB standards described in *Nucleic Acids Research* 13:3021–3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345–373 (1984) which are herein incorporated by reference.

SEQ ID NO:1 is the nucleotide sequence comprising the cDNA insert in clone bms1.pk0023.g8 encoding a maize GST.

SEQ ID NO:2 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone bms1.pk0023.g8.

SEQ ID NO:3 is the nucleotide sequence comprising the cDNA insert in clone cs.pk0010.c5 encoding a maize GST.

SEQ ID NO:4 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cs.pk0010.c5.

SEQ ID NO:5 is the nucleotide sequence comprising the cDNA insert in clone ceb1.pk0017.a5 encoding a maize GST.

SEQ ID NO:6 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone ceb1.pk0017.a5.

SEQ ID NO:7 is the nucleotide sequence comprising the cDNA insert in clone cc71se-a.pk0001.g2 encoding a maize class III GST.

SEQ ID NO:8 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cc71se-a.pk0001.g2.

SEQ ID NO:9 is the nucleotide sequence comprising the cDNA insert in clone cc71se-b.pk0014.b8 encoding a maize class III GST.

SEQ ID NO:10 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cc71se-b.pk0014.b8.

SEQ ID NO:11 is the nucleotide sequence comprising the cDNA insert in clone ceb5.pk0051.f8 encoding a maize class III GST.

SEQ ID NO:12 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone ceb5.pk0051.f8.

SEQ ID NO:13 is the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0003.b1 encoding a maize class III GST.

SEQ ID NO:14 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0003.b1.

SEQ ID NO:15 is the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0014.g8 encoding a maize class III GST.

SEQ ID NO:16 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0014.g8.

SEQ ID NO:17 is the nucleotide sequence comprising the cDNA insert in clone m.15.5.d06.sk20 encoding a maize class II GST.

SEQ ID NO:18 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone m.15.5.d06.sk20.

SEQ ID NO:19 is the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0040.e12 encoding a maize class II GST.

SEQ ID NO:20 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0040.e12.

SEQ ID NO:21 is the nucleotide sequence comprising the cDNA insert in clone ceb5.pk0049.a11 encoding a maize class III GST.

SEQ ID NO:22 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone ceb5.pk0049.a11.

SEQ ID NO:23 is the nucleotide sequence comprising the cDNA insert in clone cs1.pk0059.e2 encoding a maize class III GST.

SEQ ID NO:24 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cs1.pk0059.e2.

SEQ ID NO:25 is the nucleotide sequence comprising the cDNA insert in clone cbn2.pk0032.d10 encoding a maize class I GST.

SEQ ID NO:26 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cbn2.pk0032.d10.

SEQ ID NO:27 is the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0164.g7 encoding a maize class I GST.

SEQ ID NO:28 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0164.g7.

SEQ ID NO:29 is the nucleotide sequence comprising the cDNA insert in clone cdt2c.pk003.115 encoding a maize class I GST.

SEQ ID NO:30 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cdt2c.pk003.115.

SEQ ID NO:31 is the nucleotide sequence comprising the cDNA insert in clone csc1c.pk001.h7 encoding a maize class I GST.

SEQ ID NO:32 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone csc1c.pk001.h7.

SEQ ID NO:31 is the nucleotide sequence comprising the cDNA insert in clone csc1c.pk001.h7 encoding a maize class I GST.

SEQ ID NO:32 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone csc1c.pk001.h7.

SEQ ID NO: 33 is the nucleotide sequence comprising the cDNA insert in clone p0110.cgsnt78r encoding a maize class I GST.

SEQ ID NO:34 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0110.cgsnt78r.

SEQ ID NO:35 is the nucleotide sequence comprising the cDNA insert in clone p0121.cfrmz42r encoding a maize class I GST.

SEQ ID NO:36 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0121.cfrmz42r.

SEQ ID NO:37 is the nucleotide sequence comprising the cDNA insert in clone csi1n.pk0034.a11 encoding a maize class III GST.

SEQ ID NO:38 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone csi1n.pk0034.a11.



SEQ ID NO:39 is the nucleotide sequence comprising the cDNA insert in clone cepe7.pk0028.g3 encoding a maize class III GST.

SEQ ID NO:40 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cepe7.pk0028.g3.

SEQ ID NO:41 is the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0167.d7 encoding a maize class III GST.

SEQ ID NO:42 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cr1n.pk0167.d7.

SEQ ID NO:43 is the nucleotide sequence comprising the cDNA insert in clone cco1.pk0027.e4 encoding a maize class III GST.

SEQ ID NO:44 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cco1.pk0027.e4.

SEQ ID NO:45 is the nucleotide sequence comprising the cDNA insert in clone cpj1c.pk001.d21 encoding a maize class III GST.

SEQ ID NO:46 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cpj1c.pk001.d21.

SEQ ID NO:47 is the nucleotide sequence comprising the cDNA insert in clone cse1c.pk001.b8 encoding a maize class III GST.

SEQ ID NO:48 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cse1c.pk001.b8.

SEQ ID NO:49 is the nucleotide sequence comprising the cDNA insert in clone cr1s.pk010.f1 encoding a maize class III GST.

SEQ ID NO:50 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cr1s.pk010.f1.

SEQ ID NO:51 is the nucleotide sequence comprising the cDNA insert in clone cpf1c.pk002.a13 encoding a maize class III GST.

SEQ ID NO:52 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cpf1c.pk002.a13.

SEQ ID NO:53 is the nucleotide sequence comprising the cDNA insert in clone cho1c.pk004.c15 encoding a maize class III GST.

SEQ ID NO:54 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cho1c.pk004.c15.

SEQ ID NO:55 is the nucleotide sequence comprising the cDNA insert in clone cpi1c.pk002.m4 encoding a maize class III GST.

SEQ ID NO:56 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone cpi1c.pk002.m4.

SEQ ID NO:57 is the nucleotide sequence comprising the cDNA insert in clone chpc8.pk057.f10 encoding a maize class III GST.

SEQ ID NO:58 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone chpc8.pk057.f10.

SEQ ID NO:59 is the nucleotide sequence comprising the cDNA insert in clone p0014.ctu90r encoding a maize class III GST.

SEQ ID NO:60 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0014.ctu90r.

SEQ ID NO:61 is the nucleotide sequence comprising the cDNA insert in p0006.cbyvs55r encoding a maize class III GST.

SEQ ID NO:62 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0006.cbyvs55r.

SEQ ID NO:63 is the nucleotide sequence comprising the cDNA insert in p0037.crwaf68r encoding a maize class III GST.

SEQ ID NO:64 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0037.crwaf68r.

SEQ ID NO:65 is the nucleotide sequence comprising the cDNA insert in p0032.crcas61r encoding a maize class III GST.

SEQ ID NO:66 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0032.crcas61r.

SEQ ID NO:67 is the nucleotide sequence comprising the cDNA insert in p0088.crim45r encoding a maize class III GST.

SEQ ID NO:68 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0088.crim45r.

SEQ ID NO:69 is the nucleotide sequence comprising the cDNA insert in p0126.cnlag50r encoding a maize class III GST.

SEQ ID NO:70 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0126.cnlag50r.

SEQ ID NO:71 is the nucleotide sequence comprising the cDNA insert in p0095.cwsba73r encoding a maize class III GST.

SEQ ID NO:72 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0095.cwsba73r.

SEQ ID NO:73 is the nucleotide sequence comprising the cDNA insert in p0125.czaaj03r encoding a maize class III GST.

SEQ ID NO:74 is the deduced amino acid sequence of the nucleotide sequence comprising the cDNA insert in clone p0125.czaaj03r.

The transformed *E. coli* ceb5.pk0051.f8/pET30(LIC) BL21(DE3) containing the gene ceb5.pk0051.f8 in a pET30 (LIC) vector encoding a maize class III GST was deposited on Aug. 21, 1997 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209, U.S.A., under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The deposit is designated as ATCC 98511.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel GST nucleotide sequences and encoded proteins isolated from maize. GST enzymes are known to function in the process of detoxification of a variety of xenobiotic compounds in plants, most notably, herbicides. Nucleic acid fragments encoding at least a portion of several maize GST enzymes have been isolated and identified by comparison of random plant cDNA



sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The sequences of the present invention are useful in the construction of herbicide-tolerant transgenic plants, in the recombinant production of GST enzymes, in the development of screening assays to identify compounds inhibitory to the GST enzymes, and in screening assays to identify chemical substrates of the GSTs.

In the context of this disclosure, a number of terms shall be utilized.

As used herein "Glutathione S-Transferase" or "GST" refers to any plant derived glutathione S-transferase (GST) enzyme capable of catalyzing the conjugation of glutathione, homoglutathione and other glutathione-like analogs via a sulfhydryl group, to hydrophobic and electrophilic compounds. The term GST includes amino acid sequences longer or shorter than the length of natural GSTs, such as functional hybrid or partial fragments of GSTs, or their analogues. As used herein "GST" is not intended to be delimited on the basis of enzyme activity but may encompass amino acid sequences that possess no measurable enzyme activity but are substantially similar in to those sequences, known in the art to possess the above mentioned glutathione conjugating activity.

The term "class" or "GST class" refers to a grouping of the various GST enzymes according to amino acid identity. Currently, four classes have been identified and are referred to as "GST class I" "GST class II", "GST class III" and "GST class IV". The grouping of plant GSTs into three classes is described by Droog et al. (*Plant Physiology* 107:1139-1146 (1995)). All available amino acid sequences were aligned using the Wisconsin Genetics Computer Group package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis.), and graphically represented on a phylogenetic tree. Three groups were identified: class one including the archetypical sequences from maize GST I (X06755) and GST III (X04375); class two including the archetypical sequence from *Dianthus caryophyllus* (M64628); and class three including the archetypical sequence soybean GH2/4 (M20363). Recently, Applicants have established a further subgroup of the plant GSTs known as class IV GSTs with its archetypical sequence being In2-1 (X58573).

As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotide bases that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate alteration of gene expression by antisense or co-suppression technology or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary sequences.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% identity with the gene to be suppressed. Moreover, alterations in a gene which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded protein, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue (such as glycine) or a more hydrophobic residue (such as valine, leucine, or isoleucine). Similarly, changes which result in substitution of one negatively charged residue for another (such as aspartic acid for glutamic acid) or one positively charged residue for another (such as lysine for arginine) can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the protein molecule would also not be expected to alter the activity of the protein. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Moreover, the skilled artisan recognizes that substantially similar sequences encompassed by this invention are also defined by their ability to hybridize, under stringent conditions (0.1×SSC, 0.1% SDS, 65° C.), with the sequences exemplified herein. Preferred substantially similar nucleic acid fragments of the instant invention are those nucleic acid fragments whose DNA sequences are at least 80% identical to the DNA sequence of the nucleic acid fragments reported herein. More preferred nucleic acid fragments are at least 95% identical to the DNA sequence of the nucleic acid fragments reported herein.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a  $T_m$  of 55°, can be used, e.g., 5×SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5×SSC, 0.5% SDS. Moderate stringency hybridization conditions correspond to a higher  $T_m$ , e.g., 40% formamide, with 5× or 6×SSC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of  $T_m$  for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher  $T_m$ ) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating  $T_m$  have been derived (see Sambrook et al., supra, 9.50-9.51). For



hybridizations with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., supra, 11.7–11.8). In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferable a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides; more preferably at least about 20 nucleotides; and most preferably the length is at least 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

A “substantial portion” of an amino acid or nucleotide sequence comprising enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to putatively identify that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403–410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20–30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., in situ hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12–15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a “substantial portion” of a nucleotide sequence comprises enough of the sequence to specifically identify and/or isolate a nucleic acid fragment comprising the sequence. The instant specification teaches partial or complete amino acid and nucleotide sequences encoding one or more particular fungal proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

The term “percent identity”, as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, “identity” also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. “Identity” and “similarity” can be readily calculated by known methods, including but not limited to those described in: *Computational Molecular Biology* (Lesk, A. M., ed.) Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects* (Smith, D. W., ed.) Academic Press, New York (1993); *Computer Analysis of Sequence Data, Part I* (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, New Jersey (1994); *Sequence Analysis in Molecular Biology* (von Heinje, G., ed.) Academic Press (1987); and *Sequence Analysis Primer* (Gribskov, M. and Devereux, J., eds.) Stockton Press, New York (1991). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer

programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG Pileup program found in the GCG program package, as used in the instant invention, using the Needleman and Wunsch algorithm with their standard default values of gap creation penalty=12 and gap extension penalty=4 (Devereux et al., *Nucleic Acids Res.* 12:387–395 (1984)), BLASTP, BLASTN, and FASTA (Pearson et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:2444–2448 (1988)). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul et al., Natl. Cent. Biotechnol. Inf., Natl. Library Med. (NCBI NLM) NIH, Bethesda, Md. 20894; Altschul et al., *J. Mol. Biol.* 215:403–410 (1990)). Another preferred method to determine percent identity, is by the method of DNASTAR protein alignment protocol using the Jotun-Hein algorithm (Hein et al., *Methods Enzymol.* 183:626–645 (1990)). Default parameters for the Jotun-Hein method for alignments are: for multiple alignments, gap penalty=11, gap length penalty=3; for pairwise alignments ktuple=6. As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% “identity” to a reference nucleotide sequence of SEQ ID NO:1 it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO:1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Analogously, by a polypeptide having an amino acid sequence having at least, for example, 95% identity to a reference amino acid sequence of SEQ ID NO:2 it is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO:2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

The term “complementary” is used to describe the relationship between nucleotide bases that are capable to hybridizing to one another. For example, with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine. Accordingly, the instant invention also includes isolated nucleic acid fragments that are complementary to the complete sequences as reported in the accompanying Sequence Listing as well as those substantially similar nucleic acid sequences.



“Codon degeneracy” refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding the GST enzymes as set forth in SEQ ID Nos: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72 and SEQ ID NO:74. The skilled artisan is well aware of the “codon-bias” exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

“Synthetic genes” can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form gene segments which are then enzymatically assembled to construct the entire gene. “Chemically synthesized”, as related to a sequence of DNA, means that the component nucleotides were assembled in vitro. Manual chemical synthesis of DNA may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. “Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a DNA sequence that codes for a specific amino acid sequence. “Suitable regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influ-

ence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a DNA sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamoto and Goldberg, (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

The “translation leader sequence” refers to a DNA sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, G. D. (1995) *Molecular Biotechnology* 3:225).

The “3' non-coding sequences” refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. ((1989) *Plant Cell* 1:671-680).

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is without introns and that can be translated into protein by the cell. “cDNA” refers to a double-stranded DNA that is complementary to and derived from mRNA. “Sense” RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. “Antisense RNA” refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Pat. No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding



sequence, introns, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated yet has an effect on cellular processes.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Pat. No. 5,231,020).

"Altered levels" refers to the production of gene product (s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels, J. J., (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (supra) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (supra) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature* (London) 327:70-73; U.S. Pat. No. 4,945,050).

The term "herbicide-tolerant plant" as used herein is defined as a plant that survives and preferably grows normally at a usually effective dose of a herbicide. Herbicide tolerance in plants according to the present invention refers

to detoxification mechanisms in a plant, although the herbicide binding or target site is still sensitive.

"Thiol donor" refers to a compound that contains the structure RSH (where R is not equal to H). Within the context of the present invention suitable thiol donors may include, but are not limited to, Glutathione and homogluthathione.

"Electrophilic substrate" refers to a compound that is amenable to conjugation with glutathione or homogluthathione via a sulfhydryl group. Electrophilic substrates include a wide variety of compounds including pesticides, anti-pathogenic compounds such as fungicides and profungicides, pheromones, and herbicides. Within the context of the present invention electrophilic substrates with herbicidal activity may include, but are not limited to, chlorimuronethyl, alachlor, and atrazine, 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid, t-stilbene oxide, and 1,2-epoxy-3-(p-nitrophenoxy)propane.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

"Thiol donor" refers to a compound that contains the structure RSH (where R is not equal to H). Within the context of the present invention suitable thiol donors may include, but are not limited to, Glutathione and homogluthathione.

"Electrophilic substrate" refers to a compound that is amenable to conjugation with glutathione or homogluthathione via a sulfhydryl group. Electrophilic substrates include a wide variety of compounds including pesticides, anti-pathogenic compounds such as fungicides and profungicides, pheromones, and herbicides. Within the context of the present invention electrophilic substrates with herbicidal activity may include, but are not limited to, chlorimuronethyl, alachlor, and atrazine, 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid, t-stilbene oxide, and 1,2-epoxy-3-(p-nitrophenoxy)propane.

The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous enzymes from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other GST enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant using methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primers DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available in vitro transcription systems. In addition, specific primers can be designed and used to amplify a part of or full-length of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled



after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al., (1988) *PNAS USA* 85:8998) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al., (1989) *PNAS USA* 86:5673; Loh et al., (1989) *Science* 243:217). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman, M. A. and Martin, G. R., (1989) *Techniques* 1:165).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner, R. A. (1984) *Adv. Immunol.* 36:1; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed GST enzymes are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of GST enzyme available as well as the herbicide tolerant-phenotype of the plant.

Overexpression of the GST enzymes of the instant invention may be accomplished by first constructing chimeric genes in which the coding region are operably linked to promoters capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric genes may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals must also be provided. The instant chimeric genes may also comprise one or more introns in order to facilitate gene expression.

Any combination of any promoter and any terminator capable of inducing expression of a GST coding region may be used in the chimeric genetic sequence. Some suitable examples of promoters and terminators include those from nopaline synthase (nos), octopine synthase (ocs) and cauliflower mosaic virus (CaMV) genes. One type of efficient plant promoter that may be used is a high level plant promoter. Such promoters, in operable linkage with the genetic sequence for GST, should be capable of promoting expression of the GST such that the transformed plant is

tolerant to an herbicide due to the presence of, or increased levels of, GST enzymatic activity. High level plant promoters that may be used in this invention include the promoter of the small subunit (ss) of the ribulose-1,5-bisphosphate carboxylase from example from soybean (Berry-Lowe et al., *J. Molecular and App. Gen.*, 1:483-498 1982)), and the promoter of the chlorophyll a/b binding protein. These two promoters are known to be light-induced in plant cells (See, for example, *Genetic Engineering of Plants, an Agricultural Perspective*, A. Cashmore, Plenum, New York (1983), pages 29-38; Coruzzi, G. et al., *The Journal of Biological Chemistry*, 258:1399 (1983), and Dunsmuir, P. et al., *Journal of Molecular and Applied Genetics*, 2:285 (1983)).

Plasmid vectors comprising the instant chimeric genes can then constructed. The choice of plasmid vector depends upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., (1985) *EMBO J.* 4:2411-2418; De Almeida et al., (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA blots (Southern, *J. Mol. Biol.* 98, 503, (1975)). Northern analysis of mRNA expression (Kroczeck, *J. Chromatogr. Biomed Appl.*, 618 (1-2) (1993) 133-145), Western analysis of protein expression, or phenotypic analysis.

For some applications it will be useful to direct the instant GST enzymes to different cellular compartments or to facilitate enzyme secretion from a recombinant host cell. It is thus envisioned that the chimeric genes described above may be further supplemented by altering the coding sequences to encode enzymes with appropriate intracellular targeting sequences such as transit sequences (Keegstra, K., *Cell* 56:247-253 (1989)), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels, J. J., *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53 (1991)), or nuclear localization signals (Raikhel, N. *Plant Phys.* 100:1627-1632 (1992)) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future that are useful in the invention.

It may also be desirable to reduce or eliminate expression of the genes encoding the instant GST enzymes in plants for some applications. In order to accomplish this, chimeric genes designed for co-suppression of the instant GST enzymes can be constructed by linking the genes or gene fragments encoding the enzymes to plant promoter sequences. Alternatively, chimeric genes designed to express antisense RNA for all or part of the instant nucleic acid fragments can be constructed by linking the genes or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Plants transformed with the present GST genes will have a variety of phenotypes corresponding to the various properties conveyed by the GST class of proteins. Glutathione conjugation catalyzed by GSTs is known to result in sequestration and detoxification of a number of herbicides and other xenobiotics (Marrs et al., *Annu. Rev. Plant Physiol.*



*Plant Mol. Biol.* 47:127–58 (1996)) and thus will be expected to produce transgenic plants with this phenotype. Other GST proteins are known to be induced by various environmental stresses such as salt stress (Roxas, et al., *Stress tolerance in transgenic seedlings that overexpress glutathione S-transferase*, Annual Meeting of the American Society of Plant Physiologists, (August 1997), abstract 1574, Final Program, Plant Biology and Supplement to Plant Physiology, 301), exposure to ozone (Sharma et al., *Plant Physiology*, 105 (4) (1994) 1089–1096), and exposure to industrial pollutants such as sulfur dioxide (Navari-Izzo et al., *Plant Science* 96 (1–2) (1994) 31–40). It is contemplated that transgenic plants, tolerant to a wide variety of stresses, may be produced by the present method by expressing foreign GST genes in suitable plant hosts.

The instant GST enzymes produced in heterologous host cells, particularly in the cells of microbial hosts, can be used to prepare antibodies to the enzymes by methods well known to those skilled in the art. The antibodies are useful for detecting the enzymes in situ in cells or in vitro in cell extracts. Preferred heterologous host cells for production of the instant GST enzymes are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct chimeric genes for production of the instant GST enzymes. These chimeric genes could then be introduced into appropriate microorganisms via transformation to provide high level expression of the enzymes.

Vectors or cassettes useful for the transformation of suitable host cells are well known in the art. Typically the vector or cassette contains sequences directing transcription and translation of the relevant gene, a selectable marker, and sequences allowing autonomous replication or chromosomal integration. Suitable vectors comprise a region 5' of the gene which harbors transcriptional initiation controls and a region 3' of the DNA fragment which controls transcriptional termination. It is most preferred when both control regions are derived from genes homologous to the transformed host cell, although it is to be understood that such control regions need not be derived from the genes native to the specific species chosen as a production host.

Initiation control regions or promoters, which are useful to drive expression of the genes encoding the GST enzymes in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genes is suitable for the present invention including but not limited to *CYC1*, *HIS3*, *GAL1*, *GAL10*, *ADH1*, *PGK*, *PHO5*, *GAPDH*, *ADC1*, *TRP1*, *URA3*, *LEU2*, *ENO*, *TPI* (useful for expression in *Saccharomyces*); *AOX1* (useful for expression in *Pichia*); and *lac*, *trp*,  $\lambda P_L$ ,  $\lambda P_R$ , *T7*, *tac*, and *trc* (useful for expression in *E. coli*).

Termination control regions may also be derived from various genes native to the preferred hosts. Optionally, a termination site may be unnecessary, however, it is most preferred if included.

An example of a vector for high level expression of the instant GST enzymes in a bacterial host is provided (Example 5).

Additionally, the instant maize GST enzymes can be used as a targets to facilitate design and/or identification of inhibitors of the enzymes that may be useful as herbicides or herbicide synergists. This is desirable because the enzymes described herein catalyze the sulfhydryl conjugation of glutathione to compounds toxic to the plant. Conjugation

can result in detoxification of these compounds. It is likely that inhibition of the detoxification process will result in inhibition of plant growth or plant death. Thus, the instant maize GST enzymes could be appropriate for new herbicide or herbicide synergist discovery and design

All or a portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to expression of the instant enzymes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes or in the identification of mutants.

For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al., (1987) *Genomics* 1:174–181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al., (1980) *Am. J. Hum. Genet.* 32:314–331).

The production and use of plant gene-derived probes for use in genetic mapping are described by Bernatzky, R. and Tanksley, S. D. (*Plant Mol. Biol. Reporter* 4(1):37–41 (1986)). Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al., In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319–346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence in situ hybridization (FISH) mapping. Although current methods of FISH mapping favor use of large clones (several to several hundred KB), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification, polymorphism of PCR-amplified fragments (CAPS), allele-specific ligation, nucleotide extension reactions, Radiation Hybrid Mapping and Happy Mapping. For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence.



This, however, is generally not necessary for mapping methods. Such information may be useful in plant breeding in order to develop lines with desired starch phenotypes.

### EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

### GENERAL METHODS

Standard recombinant DNA and molecular cloning techniques used in the Examples are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, (1989) (Maniatis) and by T. J. Silhavy, M. L. Bannan, and L. W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1984) and by Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987).

#### Example 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various maize tissues were prepared. The characteristics of the libraries are described in Table 1.

TABLE 1

cDNA Libraries From Corn Tissues			
Library	GST Class	Clone	Tissue
bms1	I	bms1.pk0023.g8	Maize BMS cell culture 1 day after subculture
cs1	I	cs1.pk0010.c5	Maize leaf, sheath 5 wk plant Stratogene #837201
ceb1	I	ceb1.pk0017.a5	Maize embryo
cc71se	III	cc71se-a.pk0001.g2	Maize class II callus tissue, somatic embryo formed, highly transformable
cc71se	III	cc71se-b.pk0014.b8	Maize class II callus tissue, somatic embryo formed, highly transformable
ceb5	III	ceb5.pk0051.f8	Amplified maize embryo 30 day
cr1n	III	cr1n.pk0003.b1	Maize root from 7 day seedlings grown in light normalized
cr1n	III	cr1n.pk0014.g8	Maize root from 7 day seedlings grown in light normalized
m	II	m.15.5.d06.sk20	Maize 15 day embryo library
cr1n	II	cr1n.pk0040.e12	Maize root from 7 day seedlings grown in light normalized
ceb5	III	ceb5.pk0049.a11	Amplified maize embryo 30 day
cs1	III	cs1.pk0059.e2	Maize leaf, sheath 5 wk plant Stratogene #837201
cbn2	gst I	cbn2.pk0032.d10	Corn ( <i>Zea mays</i> L.) developing kernel two days after pollination
cr1n	gst I	cr1n.pk0164.g7	Corn ( <i>Zea mays</i> L.) root from 7 day seedlings grown in light normalized

TABLE 1-continued

cDNA Libraries From Corn Tissues			
Library	GST Class	Clone	Tissue
cdt2	gst I	cdt2c.pk003.l15	Corn ( <i>Zea mays</i> L.) developing tassel 2
csc1c	gst I	csc1c.pk001.h7	Corn ( <i>Zea mays</i> L., B73) 20 day seedling (germination cold stress). The seedling appeared purple.
p0110	gst I	p0110.cgsnt78r	Corn ( <i>Zea mays</i> L. B73) salicylic acid infiltrated V3/V4 leaf tissue (minus midrib), screened 1 pool of A63 + SA 4 h; A63 + SA 24 hr; and A63 + SA 7 days
p0121	gst I	p0121.cfrmz42r	Corn ( <i>Zea mays</i> L.) shank tissue collected from ears 5DAP, Screened 1
csi1n	gst III	csi1n.pk0034.a11	Corn ( <i>Zea mays</i> L.) silk; normalized from csi1 library
cepe7	gst III	cepe7.pk0028.g3	Corn ( <i>Zea mays</i> L.) epicotyl from 7 day old etiolated seedling
cr1n	gst III	cr1n.pk0167.d7	Corn ( <i>Zea mays</i> L.) root from 7 day seedlings grown in light normalized
cco1	gst III	cco1.pk0027.e4	Corn ( <i>Zea mays</i> L.) cob of 67 day old plants grown in green house
cpj1c	gst III	cpj1c.pk001.d21	Corn ( <i>Zea mays</i> L.) pooled black mexican sweetcorn treated with chemicals related to membrane ionic force
cse1c	gst III	cse1c.pk001.b8	Corn ( <i>Zea mays</i> L.) seedling at V2 stage treated with Ethylene collected at 6 hr, 23 hr, 72 hr
cr1s	gst III	cr1s.pk010.f1	Corn ( <i>Zea mays</i> L., Lh132) root from 7 day old etiolated seedlings
cpf1c	gst III	cpf1c.pk002.a13	Corn ( <i>Zea mays</i> L.) pooled black mexican sweetcorn treated with chemicals related to protein synthesis
cho1c	gst III	cho1c.pk004.c15	Corn ( <i>Zea mays</i> L., Alexho Synthetic High Oil) embryo 20 DAP
cpi1c	gst III	cpi1c.pk002.m4	Corn ( <i>Zea mays</i> L.) pooled black mexican sweetcorn treated with chemicals related to biochemical compound synthesis
chpc8	gst III	chpc8.pk057.f10	Corn ( <i>Zea mays</i> L., MBS847) 8 day old shoot treated with PDO herbicide MK593 collected 8 hrs after treatment.
p0014	gst III	p0014.ctu90r	Corn ( <i>Zea mays</i> L.) Leaf: Gene uaz151 (G-Protein), 413-8, no genetic lesions are formed. <i>C. heterostrophus</i> resistance, plant 3 ft tall, leaf 7 and leaf 8
p0006	gst III	p0006.cbyvs55r	Corn ( <i>Zea mays</i> L.) Young shoot
p0037	gst III	p0037.crwaf68r	Corn ( <i>Zea mays</i> L.) corn Root Worm infested V5 roots
p0032	gst III	p0032.crcas61r	Corn ( <i>Zea mays</i> L.) Regenerating callus, 10 and 14 days after auxin removal. Hi-II callus 223a, 1129e 10 days. Hi-II callus 223a, 1129e 14 days
p0088	gst III	p0088.c1rim45r	Corn ( <i>Zea mays</i> L.) Gene M1C07 (leucine-rich repeat), family 3-B7. about one month after planting in green house
p0126	gst III	p0126.cn1ag50r	Corn ( <i>Zea mays</i> L.) Night harvested leaf tissue; V8-V10
p0095	gst III	p0095.cwsba73r	Corn ( <i>Zea mays</i> L.) Ear leaf sheath, screened 1 Growth conditions: field; control or



TABLE 1-continued

cDNA Libraries From Corn Tissues			
Library	GST Class	Clone	Tissue
p0125	gst III	p0125.czaaj03r	untreated tissues Growth stage: 2-3 weeks after pollen shed; plants were allowed to pollinate naturally Corn ( <i>Zea mays</i> L.) Anther: Prophase I screened 1

cDNA libraries were prepared in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, Calif.). The Uni-ZAP™ XR libraries were converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts were contained in the plasmid vector pBluescript. cDNA inserts from randomly picked bacterial colonies containing recombinant pBluescript plasmids were amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs were sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams, M. D. et al., (1991) *Science* 252:1651). The resulting ESTs were analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

#### Example 2

##### Identification and Characterization of cDNA Clones

cDNAs encoding maize GST enzymes were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) searches for similarity to sequences contained in the BLAST "nr" database

(comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) *Nature Genetics* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

All comparisons were done using either the BLASTNnr or BLASTXnr algorithms. The results of the BLAST comparisons are given in Table 2 and summarize the clones and the sequences to which they have the most similarity. Table 2 displays data based on the BLASTNnr or BLASTXnr algorithm with values reported in pLogs or Expect values. The Expect value estimates the statistical significance of the match, specifying the number of matches, with a given score, that are expected in a search of a database of this size absolutely by chance. Each cDNA identified encodes at least a portion of either a GST class I, II, or III. All isolated clones contain a full length open reading frame (ORF) with the exception of cc71se-a.pk0001.g2 which is only a partial clone. Example 5 describes the sequencing strategy for the above described clones.

TABLE 2

BLAST Results For Clones							
SEQ ID NO.							
Clone	GST Class	Similarity Identified	Base	Peptide	Blast Algorithm	pLog Score*/E-Value**	Citation
bms1.pk0023.g8	I	X79515 ZMGST27 <i>Z. mays</i> GST-27 mRNA for glutathione-S-transferase	1	2	Nnr	122.086	
cs1.pk0010.c5	I	D17673 ATHERD13 <i>Arabidopsis thaliana</i> mRNA for glutathione S-transferase	3	4	Nnr	8.16	
ceb1.pk0017.a5	I	X78203 HMGST <i>H. muticus</i> mRNA for glutathione S-transferase	5	6	Nnr	21.51	
cc71se-a.pk0001.g2	III	(AF004358) glutathione S-transferase TSI-1 ( <i>Aegilops squarrosa</i> )	7	8	Nnr	16.48	
cc71se-b.pk0014.b8	III	D10861 RICORFC Rice mRNA for a protein related to chilling tolerance.	9	10	Nnr	14.96	
ceb5.pk0051.f8	III	D1086T RICORFC Rice mRNA for a protein related to chilling tolerance.	11	12	Nnr	40.44	
cr1n.pk0003.b1	III	U80615 EGU80615 Eucalyptus globulus auxin-induced protein (EgPar) mRNA, complete cds	13	14	Nnr	24.70	
cr1n.pk0014.g8	III	M16901 MZEGSTIB Maize glutathione S-transferase (GST-I) mRNA, complete cds	15	16	Nnr	5.85	
m.15.5.d06.sk20	II	M97702 DROGLUSTD <i>Drosophila melanogaster</i> glutathione S-transferase gene.	17	18	Nnr	3.63	
cr1n.pk0040.e12	II	167970 (L05915) (GST1) gene	19	20	Xnr	42.03	



TABLE 2-continued

Clone	GST Class	Similarity Identified	BLAST Results For Clones SEQ ID NO.			pLog Score*/E-Value**	Citation
			Base	Peptide	Blast Algorithm		
ceb5.pk0049.a11	III	product ( <i>Dianthus caryophyllus</i> )  Y12862 ZYMY12862 Zea Maize mRNA for glutathione S-transferase	21	22	Nnr	0.0	
cs1.pk0059.e2	III	D10861 RICORFC Rice mRNA for a protein related to chilling tolerance.	24	25	Nnr	41.03	
cbn2.pk0032.d10	gst I	(AC005309) glutathione s-transferase, GST6 ( <i>Arabidopsis thaliana</i> )	25	26	Xnr	4e-27	unpublished
cr1n.pk0164.g7	gst I	(AC005309) glutathione s-transferase, GST6 [ <i>Arabidopsis thaliana</i> ]	27	28	Xnr	7e-37	unpublished
cdec.pk003.115	gst I	(AC005309) glutathione s-transferase, GST6 [ <i>Arabidopsis thaliana</i> ]	29	30	Xnr	4e-36	unpublished
csc1c.pk001.h7	gst I	(U70672) glutathione S-transferase [ <i>Arabidopsis thaliana</i> ]	31	32	Xnr	8e-34	unpublished
p0110.cgsnt78r	gst I	P46420 GTH4_MAIZE GLUTSTHION S-TRANSFERASE IV (GST-IV) (GS-27)	33	34	Xnr	1e-97	Plant Mol. Biol. 26 (6), 1855-1866 (1994)
p0121.cfrmz42r.	gst I	P42761 GTH3_ARATH GLUTETHIONE S-TRANSFERASE ERD13 (CLASS PHI)	35	36	Xnr	3e-28	FEBS Lett. 335 (2), 189-192 (1993)
csi1n.pk0034.a11	gst III	Q03664 GTX3_TOBAC PROBABLE GLUTATHIONE S-TRANSFERASE (AUXIN-INDUCED PROTEIN PCNT103)	37	38	Xnr	2e-51	Plant Mol. Biol. 16 (6), 983-998 (1991)
cepe7.pk0028.g3	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	39	40	Xnr	9e-50	Plant Physiol. 114, 1461-1470 (1997)
cr1n.pk0167.d7	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	41	42	Xnr	8e-58	Plant Physiol. 114, 1461-1470 (1997)
cco1.pk0027.e4	gst III	(Y12862) glutathione transferase [ <i>Zea mays</i> ]	43	44	Xnr	2e-77	JOURNAL Plant Mol. Biol. 36, 75-87 (1998)
cpj1c.pk001.d21	gst III	Q03662 GTX1_TOBAC PROBABLE GLUTATHIONE S-TRANSFERASE (AUXIN- INDUCED PROTEIN PGNT1/PCNT110)	45	46	Xnr	1e-53	Plant Mol. Biol. 16 (6), 983-998 (1991)
cse1c.pk001.b8	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	47	48	Xnr	3e-62	Plant Physiol. 114, 1461-1470 (1997)
cr1s.pk010.f1	gst III	P32110 GTX6_SOYBN PROBABLE GLUTATHIONE S-TRANSFERASE (HEAT SHOCK PROTEIN 26A)	49	50	Xnr	4e-49	Mol. Cell. Biol. 8 (3), 1113-1122 (1988)
cpf1c.pk002.a13	gst III	Q03662 GTX1_TOBAC PROBABLE GLUTATHIONE S-TRANSFERASE (AUXIN-INDUCED PROTEIN PGNT1/PCNT110)	51	52	Xnr	6e-47	Plant Mol. Biol. 16 (6), 983-998 (1991)
cho1c.pk004.c15	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	53	54	Xnr	1e-59	Plant Physiol. 114, 1461-1470 (1997)
cpi1c.pk002.m4	gst III	(AF051214) probable glutathione S-transferase [ <i>Picea mariana</i> ]	55	56	Xnr	7e-45	Genetics 149 (2), 1089-1098 (1998)
chpc8.pk057.f10	gst III	(AJ010449) glutathione transferase [ <i>Alopecurus myosuroides</i> ]	57	58	Xnr	1e-62	unpublished
p0014.ctu90r	gst III	(AJ010448) glutathione transferase [ <i>Alopecurus myosuroides</i> ]	59	60	Xnr	8e-55	unpublished
p0006.cbyvs55r	gst III	(AF051214) probable glutathione S-transferase [ <i>Picea mariana</i> ]	61	62	Xnr	4e-51	Genetics 149 (2), 1089-1098 (1998)
p0037.crwaf68r	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	63	64	Xnr	2e-77	Plant Physiol. 114, 1461-1470 (1997)
p0032.crcas61r	gst III	P32110 GTX6_SOYBN PROBABLE GLUTATHIONE S-TRANSFERASE (HEAT SHOCK PROTEIN 26A)	65	66	Xnr	3e-48	Mol. Cell. Biol. 8 (3), 1113-1122 (1988)
p0088.c1rim45r	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	67	68	Xnr	3e-53	Plant Physiol. 114, 1461-1470 (1997)
p0126.cn1ag50r	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	69	70	Xnr	4e-52	Plant Physiol. 114, 1461-1470 (1997)
p0095.cwsba73r	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	71	72	Xnr	2e-57	Plant Physiol. 114, 1461-1470 (1997)
p0125.czaaj03r	gst III	(AF004358) glutathione S-transferase TSI-1 [ <i>Aegilops squarrosa</i> ]	73	74	Xnr	7E-63	Plant Physiol. 114, 1461-1470 (1997)

\*Plog represents the negative of the logarithm of the reported P-value

\*\*Expect value estimates the statistical significance of the match, specifying the number of matches, with a given score, that are expected in a search of a database of this size absolutely by chance.



## Example 3

## Expression of Chimeric Genes Encoding Maize GST Enzymes in Maize Cells (Monocotyledon)

A chimeric gene comprising a cDNA encoding a maize GST enzyme in sense orientation can be constructed by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a 100  $\mu$ L volume in a standard PCR mix consisting of 0.4 mM of each oligonucleotide and 0.3 pM of target DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 mM dGTP, 200 mM dATP, 200 mM dTTP, 200 mM dCTP and 0.025 unit DNA polymerase. Reactions are carried out in a Perkin-Elmer Cetus Thermocycler™ for 30 cycles comprising 1 min at 95° C., 2 min at 55° C. and 3 min at 72° C., with a final 7 min extension at 72° C. after the last cycle. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on a 0.7% low melting point agarose gel in 40 mM Tris-acetate, pH 8.5, 1 mM EDTA. The appropriate band can be excised from the gel, melted at 68° C. and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega Corp 7113 Benhart Dr, Raleigh, N.C.). Vector and insert DNA can be ligated at 15° C. overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (*Epicurian Coli* XL-1; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a plant GST enzyme, and the 10 kD zein 3' region. The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132 (Indiana Agric. Exp. Station, Ind., USA). The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., (1975) *Sci. Sin.* Peking 18:659-668). The embryos are kept in the dark at 27° C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks. The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, v Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the Pat gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The pat gene in p35S/Ac is under the

control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3M region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The particle bombardment method (Klein et al., (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles ((1  $\mu$ m in diameter) are coated with DNA using the following technique. Ten  $\mu$ g of plasmid DNAs are added to 50  $\mu$ L of a suspension of gold particles (60 mg per mL). Calcium chloride (50  $\mu$ L of a 2.5 M solution) and spermidine free base (20  $\mu$ L of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200  $\mu$ L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30  $\mu$ L of ethanol. An aliquot (5  $\mu$ L) of the DNA-coated gold particles can be placed in the center of a flying disc (Bio-Rad Labs, 861 Ridgeview Dr, Medina, Ohio). The particles are then accelerated into the corn tissue with a PDS-1000/He (Bio-Rad Labs, 861 Ridgeview Dr, Medina, Ohio), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm. For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi. Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium. Plants can be regenerated from tie transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al., (1990) *Bio/Technology* 8:833-839).

## Example 4

## Expression of Chimeric Genes in Tobacco Cells (Dicotyledon)

Cloning sites (XbaI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pBI121 (Clonetech Inc., 6500 Donlon Rd, Somis, Calif.) or other appropriate transformation vector. Amplification could be performed as described above and the amplified DNA would then be digested with restriction enzymes XbaI and SmaI and fractionated on a 0.7% low melting point agarose gel in 40 mM-Tris-acetate, pH 8.5, 1 mM EDTA. The appropriate band can be excised from the gel, melted at 68° C. and combined with a 13 kb XbaI-SmaI fragment of the plasmid pBI121 and handled as in Example 3. The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, right border region, the nos promoter



linked to the NPT II gene and a nos terminator region followed by a cauliflower mosaic virus 35S promoter linked to a cDNA fragment encoding a plant GST enzyme and the nos terminator 3' region flanked by the left border region. The resulting plasmid could be mobilized into the *Agrobacterium* strain LBA4404/pAL4404 (Hoekema et al. *Nature* 303:179–180, (1983) using triparental matings (Ruvkin and Ausubel, *Nature* 289:85–88, (1981)). The resulting *Agrobacterium* strains could be then cocultivated with protoplasts (van den Elzen et al. *Plant Mol. Biol.* 5:149–154 (1985)) or leaf disks (Horsch et al. *Science* 227:1229–1231, (1985)) of *Nicotiana tabacum* cv Wisconsin 38 and kanamycin-resistant transformants would be selected. Kanamycin-resistant transformed tobacco plants would be regenerated.

#### Example 5

##### Expression of Chimeric Genes in Microbial Cells and Purification of Gene Product

Example 5 illustrates the expression of isolated full length genes encoding either class I, II or III GST proteins in *E. coli*.

All clones listed in Table 2 were selected on the basis of homology to known GSTs using the BLAST algorithm as described in Example 2. Plasmid DNA was purified using QIAFilter cartridges (Qiagen, Inc., 9600 De Soto Ave, Chatsworth, Calif.) according to the manufacturer's instructions. Sequence was generated on an ABI Automatic sequencer using dye terminator technology (U.S. Pat. No. 5,366,860; EP 272007) using a combination of vector and insert-specific primers. Sequence editing was performed in either DNASTar (DNA, Star Inc.) or the Wisconsin GCG program (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis.). All sequences represent coverage at least two times in both directions.

cDNA from the clones bms1.pk0023.g8, cs1.pk0010.c5, ceb1.pk0017.a5, m.15.5.d06.sk20, ceb5.pk0049.a11, ceb5.pk0051.f8, and cs1.pk0059.e2, encoding the instant maize GST enzymes were inserted into the ligation independent cloning (LIC) pET30 vector (Novagen, Inc., 597 Science Dr, Madison, Wis.) under the control of the T7 promoter, according to the manufacturer's instructions (see Novagen publications "LIC Vector Kits", publication number TB163 and U.S. Pat. No. 4,952,496). The vector was then used to transform BL21(DE3) competent *E. coli* hosts. Primers with a specific 3' extension designed for ligation independent cloning were designed to amplify the GST gene (Maniatis). Amplification products were gel-purified and annealed into the LIC vector after treatment with T4 DNA polymerase (Novagen). Insert-containing vectors were then used to transform NovaBlue competent *E. coli* cells and transformants were screened for the presence of viable inserts. Clones in the correct orientation with respect to the T7 promoter were transformed into BL21(DE3) competent cells (Novagen) and selected on LB agar plates containing 50 µg/mL kanamycin. Colonies arising from this transformation were grown overnight at 37° C. in Lauria Broth to OD 600=0.6 and induced with 1 mM IPTG and allowed to grow for an additional two hours. The culture was harvested, resuspended in binding buffer, lysed with a French press and cleared by centrifugation.

Expressed protein was purified using the HIS binding kit (Novagen) according to the manufacturer's instructions.

Purified protein was examined on 15–20% SDS Phast Gels (Bio-Rad Laboratories, 861 Ridgeview Dr, Medina, Ohio) and quantitated spectrophotometrically using BSA as a standard. Protein data is tabulated below in Table 3.

TABLE 3

Protein Expression Data	
CLONE	OD.280
bms1.pk0023.g8	0.57
cs1.pk0010.c5	0.53
ceb1.pk0017.a5	0.50
m.15.5.d06.sk20	0.39
ceb5.pk0049.a11	2.06
ceb5.pk0051.f8	1.30
cs1.pk0059.e2	1.45

#### Example 6

##### Screening of Expressed GST Enzymes for Substrate Metabolism

The GST enzymes, expressed and purified as described in Example 5 were screened for their ability to metabolize a variety of substrates. Substrates tested included the three herbicide electrophilic substrates chlorimuron ethyl, alachlor, and Atrazine, and four model electrophilic substrates, 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid, t-stilbene oxide, and 1,2-epoxy-3-(p-nitrophenoxy) propane. The enzymes were purified as described in Example 5 and used in the following assay.

For each enzyme, the conjugation reaction with each electrophilic substrate was performed by incubating 0.3 to 30 µg enzyme in 0.1 M MOPS (pH 7.0) containing 0.4 mM of the electrophilic substrate. The reaction was initiated by the addition of glutathione to a final concentration of 4 mM. After 5 to 30 min, the reaction was terminated by the addition of 45 µL acetonitrile, microfuged for 10 min to remove precipitated protein, and then the supernatant was removed and added to 65 µl of water. This sample was chromatographed on a Zorbax C8 reverse phase HPLC column (3 µm particle size, 6.2 mm×8 cm) using a combination of linear gradients (flow=1.5 mL/min) of 1% H<sub>3</sub>PO<sub>4</sub> in water (solvent A) and 1% H<sub>3</sub>PO<sub>4</sub> in acetonitrile. The gradient started with 5% solvent B, progressing from 5% to 75% solvent B between 1 and 10 min, and from 75% to 95% solvent B between 10 and 12 min. Control reactions without enzyme were performed to correct for uncatalyzed reaction. Quantitation of metabolites were based on an assumption that the extinction coefficient of the conjugate was identical to that of the electrophilic substrate.

Table 4 shows the activity of each enzyme measured in nmol.min<sup>-1</sup>.mg<sup>-1</sup> with the seven different substrates. Activities are related to the activities of the known and previously isolated and purified GST enzymes, BZ-II (Marrs et al., *Nature* 375:397–400 (1995)), pIN2-1 (Hershey et al., *Plant Molecular Biology* 17:679–690, (1991)), GST-I, GST-III, and GST-IV, collectively described in Shah et al., *Plant Mol Biol* 6, 203–211(1986); Jepson et al., *Plant Mol Biol* 26:1855–1866, (1994); Moore et al., *Nucleic Acids Res* 14:7227–7235 (1986); and Holt et al., *Planta* 196:295–302, (1995).



TABLE 4

GST Name	GST Class	Chlor- Imuron- Ethyl	Alachlor	Atrazine	CDNB	Ethacrynic Acid	t-Stilbene Oxide	1,2-epoxy-3- (p-nitrophenoxy) propane
cs1.pk0059.e2	III	0.1	8	0.02	1348	20	1.25	43
ceb5.pk0049.a11	III	0.4	18	0.01	3939	102	0.01	30
ceb5.pk0051.f8	III	1.9	27	0.08	2136	117	0.02	14
BZ-II	III	0.2	0	0.00	15	23	0.05	0
ceb1.pk0017.a5	I	0.1	0	0.00	15	5	0.00	0
cs1.pk0010.c5	I	0.1	0	0.00	30	9	0.00	0
bms1.pk0023.g8	I	0.2	0	0.00	15	13	0.00	0
GST-IV	i	0.3	1	0.00	15	13	0.00	0
GST-I	I	0.4	77	0.60	46485	32	0.98	92
GST-III	I	0.3	3	0.05	1803	1	0.31	28
m.15.5.d06.sk20	II	0.1	0	0.00	45	17	0.00	1
pIN2-1	IV	0	0	—	15	—	—	—

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Thr	Leu	Tyr	Gln	Ser	Arg	Ala	Ile	Ala	Arg	Tyr	Val	Leu	Arg	Lys	Leu
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Lys	Pro	Glu	Leu	Leu	Arg	Glu	Gly	Asp	Leu	Glu	Gly	Ser	Ala	Met	Val
				85					90					95	
Asp	Ala	Trp	Met	Glu	Val	Glu	Ala	His	His	Met	Glu	Pro	Ala	Leu	Trp
			100						105					110	
Pro	Ile	Ile	Arg	His	Ser	Ile	Ile	Gly	Gln	Tyr	Val	Gly	Arg	Glu	Arg
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Asp	His	Gln	Ala	Val	Ile	Asp	Glu	Asn	Leu	Asp	Arg	Leu	Arg	Lys	Val
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Leu	Pro	Ala	Tyr	Glu	Ala	Arg	Leu	Ser	Val	Cys	Lys	Tyr	Leu	Val	Gly
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Asp	Asp	Ile	Ser	Ala	Ala	Asp	Leu	Cys	His	Phe	Gly	Phe	Met	Arg	Tyr
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Phe	Met	Ala	Thr	Glu	Tyr	Ala	Gly	Leu	Val	Asp	Ala	Tyr	Pro	His	Val
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Lys	Ala	Trp	Trp	Asp	Ala	Leu	Leu	Ala	Arg	Pro	Ser	Val	Gln	Lys	Val
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 Thr Leu Val Asp Ser Arg Asp Ile Cys Arg Tyr Val Cys Asn Gln Phe  
 65 70 75 80  
 Pro Asn Tyr Gly Asn Lys Ser Leu Tyr Gly Ser Gly Ala Leu Glu Arg  
 85 90 95  
 Ala Ser Ile Glu Gln Trp Leu Gln Ala Glu Ala Gln Asn Phe Gly Pro  
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 115 120 125  
 His Leu Gly Val Arg Gln Asp Pro Ala Val Ile Ala Glu Asn Glu Asp  
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 Lys Leu Lys Gln Val Leu Asp Val Tyr Asp Glu Ile Leu Ser Lys Asn  
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 Glu Tyr Leu Ala Gly Asp Glu Phe Thr Leu Ala Asp Leu Ser His Leu  
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Lys Leu Gln Pro Phe Gly Gln Val Pro Ala Phe Lys Asp His Leu Thr
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Thr Val Phe Glu Ser Arg Ala Ile Cys Arg Tyr Ile Cys Asp Gln Tyr
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Ala Asp Ser Gly Asn Gln Ala Leu Phe Gly Lys Lys Glu Asp Gly Ala
          85             90             95
Val Gly Arg Ala Ala Ile Glu Gln Trp Ile Glu Ser Glu Gly Gln Ser
          100            105            110
Phe Asn Pro Pro Ser Leu Ala Ile Ile Phe Gln Leu Ala Phe Ala Pro
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          130            135            140
Lys Leu Ala Lys Val Leu Asp Val Tyr Asp Gln Arg Leu Gly Glu Ser
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Gln Thr Val Met Pro Asp Val Gly Arg Leu Leu Glu Phe Gly Lys Ala
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acgcgttccg cgcggaggcg gcgctgtgcc tgaagggcgt gccgtacgag ctgatcctgg 120
aggacctggt cggcagcaag agcgagctcc tgctccacca caaccccgtg cacaagaagg 180
tgcccgtgct cctccacggc gacggccggg ccattctccga gtccctcgtc atcgccgagt 240
acgtcgacga ggccttcgac gggccgccgc tgctccccgc cgaccctac gcgcgcgccg 300
ccgcccgctt ctgggccgac ttcattcgaga ccaggctcac caagcccttc ttcattggcg 360
tctgggtgga ggagcgcgac gcgcggctgc ggttcgagga ggaggccaag gagctcgtgg 420
cgctgctgga ggcgcagctc gagggaaaga ggttcttcgc cggcgacagg ccgggggtacc 480
tcgacgtggc cgcgtccgcy ctcgggccct ggcgacagct catcgaggag ctcaacggtg 540
tggcgctgct cagcgaggat gaccacccca acctgtgccc gtggaccagg gactactgcy 600
ccttcgaggc tctcaagccg tgcattgccg atcgggagaa gtcctcgcct tacttcacta 660
agaacttcga caggtacaag gcggccgtca atgcgacgct atcgagtcg cagcagtaat 720
aactgcccc a ctgggtacgc ctctgcccgg ccgtatggcg ggcgtttctt tttttcttc 780
ttcagaataa cgtagctgtg ccagctactc atgttttcaa ttctgcaaag tgcaaacc aa 840
caagtcgctg tgtggtttac tctttttaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 900
aaaaaaaaa a 911
```

```
<210> SEQ ID NO 10
<211> LENGTH: 235
<212> TYPE: PRT
<213> ORGANISM: maize
```

```
<400> SEQUENCE: 10
```

```
Met Ser Ser Pro Pro Pro Val Lys Leu Ile Gly Phe Phe Gly Ser Pro
  1           5           10           15
Tyr Ala Phe Arg Ala Glu Ala Ala Leu Cys Leu Lys Gly Val Pro Tyr
           20           25           30
Glu Leu Ile Leu Glu Asp Leu Phe Gly Ser Lys Ser Glu Leu Leu Leu
```



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35	40	45	
His His Asn Pro Val His Lys Lys Val Pro Val Leu Leu His Gly Asp			
50	55	60	
Gly Arg Ala Ile Ser Glu Ser Leu Val Ile Ala Glu Tyr Val Asp Glu			
65	70	75	80
Ala Phe Asp Gly Pro Pro Leu Leu Pro Ala Asp Pro Tyr Ala Arg Ala			
	85	90	95
Ala Ala Arg Phe Trp Ala Asp Phe Ile Glu Thr Arg Leu Thr Lys Pro			
	100	105	110
Phe Phe Met Ala Ile Trp Val Glu Glu Arg Asp Ala Arg Leu Arg Phe			
	115	120	125
Glu Glu Glu Ala Lys Glu Leu Val Ala Leu Leu Glu Ala Gln Leu Glu			
	130	135	140
Gly Lys Arg Phe Phe Ala Gly Asp Arg Pro Gly Tyr Leu Asp Val Ala			
	145	150	155
Ala Ser Ala Leu Gly Pro Trp Arg Ser Val Ile Glu Glu Leu Asn Gly			
	165	170	175
Val Ala Leu Leu Ser Glu Asp Asp His Pro Asn Leu Cys Arg Trp Thr			
	180	185	190
Arg Asp Tyr Cys Ala Phe Glu Ala Leu Lys Pro Cys Met Pro Asp Arg			
	195	200	205
Glu Lys Leu Leu Ala Tyr Phe Thr Lys Asn Phe Asp Arg Tyr Lys Ala			
	210	215	220
Ala Val Asn Ala Thr Leu Ser Gln Ser Gln Gln			
	225	230	235

<210> SEQ ID NO 11  
 <211> LENGTH: 948  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 11

```

agcgcacatgca ggtagcaatg gcgggggaga cgaagaaggg cctggtgctg ctggacttct    60
gggtgagccc gttcgggcag cgctgccgca tocgctggc ggagaagggc atcgcttacg    120
agtactcgga gcaggagctg ctgggcggcg ccaagagcga catcctcctc cgctccaacc    180
cgggtgcacaa gaagatcccc gtgctcctcc acgacggccg ccccgctctgc gactccctcg    240
tcacctcga gtacctcgag gaggccttcc cggaggcctc cccaggctg ctccccgacg    300
ccgcctacgc gcgcgcgcag gcccgcttct gggcggccta ctccgacaag gtctacaagg    360
ccggcacgcg gctgtggaag ctcaggggcg acgcgcgggc gcaggcgcgc gccgagatcg    420
tgcagggtgt ccggaacctc gacggcgagc taggggacaa ggccttcttc ggcggcgagg    480
cgttcgggtt cgtggacgtg gcgctcgtgc ccttcgtgcc gtggctcccc agctacgagc    540
ggtacgggga cttcagcgtg gcggagatcg cgcccaggct ggcggcgtgg gcgcgcccgt    600
gcgcgcagcg ggagagcgtg gccaggaccc ttcacccgcc ggaaaagggtg gacgagttca    660
tcaacctgct caagaagacc tacggcatcg agtagtagag cggactacta ctagcagagg    720
agatggtacc ggccgtacgt acgtggctgc catgcagttt ttgtttcggg ttgtttaaac    780
gggactccat gaatggatgg aactcttctt gggctccgtg tgctacatac acatctgtaa    840
aggtgaacta aatcacggt aaaaactcgg aaattagttt gtaaagggtc cagccccct    900
cctttataaa tagagagta tacggctgat aaaaaaaaaa aaaaaaaa    948
    
```



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<210> SEQ ID NO 12  
 <211> LENGTH: 225  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 12

Met Ala Gly Glu Thr Lys Lys Gly Leu Val Leu Leu Asp Phe Trp Val  
 1 5 10 15  
 Ser Pro Phe Gly Gln Arg Cys Arg Ile Ala Leu Ala Glu Lys Gly Ile  
 20 25 30  
 Ala Tyr Glu Tyr Ser Glu Gln Glu Leu Leu Gly Gly Ala Lys Ser Asp  
 35 40 45  
 Ile Leu Leu Arg Ser Asn Pro Val His Lys Lys Ile Pro Val Leu Leu  
 50 55 60  
 His Asp Gly Arg Pro Val Cys Glu Ser Leu Val Ile Leu Glu Tyr Leu  
 65 70 75 80  
 Glu Glu Ala Phe Pro Glu Ala Ser Pro Arg Leu Leu Pro Asp Ala Ala  
 85 90 95  
 Tyr Ala Arg Ala Gln Ala Arg Phe Trp Ala Ala Tyr Ser Asp Lys Val  
 100 105 110  
 Tyr Lys Ala Gly Thr Arg Leu Trp Lys Leu Arg Gly Asp Ala Arg Ala  
 115 120 125  
 Gln Ala Arg Ala Glu Ile Val Gln Val Val Arg Asn Leu Asp Gly Glu  
 130 135 140  
 Leu Gly Asp Lys Ala Phe Phe Gly Gly Glu Ala Phe Gly Phe Val Asp  
 145 150 155 160  
 Val Ala Leu Val Pro Phe Val Pro Trp Leu Pro Ser Tyr Glu Arg Tyr  
 165 170 175  
 Gly Asp Phe Ser Val Ala Glu Ile Ala Pro Arg Leu Ala Ala Trp Ala  
 180 185 190  
 Arg Arg Cys Ala Gln Arg Glu Ser Val Ala Arg Thr Leu His Pro Pro  
 195 200 205  
 Glu Lys Val Asp Glu Phe Ile Asn Leu Leu Lys Lys Thr Tyr Gly Ile  
 210 215 220  
 Glu  
 225

<210> SEQ ID NO 13  
 <211> LENGTH: 840  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 13

gttggggatg tgggcgagcc ctatggtgat caggggtggag tgggcgctgc ggctgaaggg 60  
 cgtcgagtac gagtacgtcg acgaggacct cgccaacaag agcgccgacc tgctccgcca 120  
 caaccgggtg accaagaagg tgcccgtgct cgtccacgac ggcaagccgg tcgcgagtc 180  
 caccatcatc gtcgagtaca tcgacgaggt ctggaagggc ggctaccca tcatgccggg 240  
 cgaccctac gagcgtgccc aggcaaggtt ctgggccagg ttcgctgaag acaagtgcaa 300  
 cgctgctctg taccgatct tcaccgac cggcgaggcg cagcgcaagg cgggtcacga 360  
 ggcccagcag tgctcaaga ccctggagac ggccttgac gggaagaagt tcttcggcgg 420  
 ggacgccgtg ggctacctcg acatcgtcgt cgggtggttc gcgcactggc tccccgtcat 480  
 cgaggaggtg accggcgcca gcgtcgtcac cgacgaggag ctgccgctga tgaaggcctg 540  
 gttcggccgg ttctcgcgg ttgacgtggt gaaggcggcc ctgcccgaca gggacaggct 600



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```

cctcgccgcc aacaaggccc gccgtgagca gctcctctcc gcgtagatgg ctagtaattc 660
tggagcagct agtttcaccg ccgacgctca tatattgctg aataaggact ggttgcaact 720
ttgcacgttg tgcaagtgcag ccgaggtttg gatgacctct gccctctgt tccatttcag 780
aatggtagtc ccataataat gcatatacat catgcataaa aaaaaaaaaa aaaaaaaaaa 840

```

```

<210> SEQ ID NO 14
<211> LENGTH: 212
<212> TYPE: PRT
<213> ORGANISM: maize

```

```

<400> SEQUENCE: 14

```

```

Met Trp Ala Ser Pro Met Val Ile Arg Val Glu Trp Ala Leu Arg Leu
 1             5             10            15
Lys Gly Val Glu Tyr Glu Tyr Val Asp Glu Asp Leu Ala Asn Lys Ser
             20            25            30
Ala Asp Leu Leu Arg His Asn Pro Val Thr Lys Lys Val Pro Val Leu
             35            40            45
Val His Asp Gly Lys Pro Val Ala Glu Ser Thr Ile Ile Val Glu Tyr
             50            55            60
Ile Asp Glu Val Trp Lys Gly Gly Tyr Pro Ile Met Pro Gly Asp Pro
             65            70            75            80
Tyr Glu Arg Ala Gln Ala Arg Phe Trp Ala Arg Phe Ala Glu Asp Lys
             85            90            95
Cys Asn Ala Ala Leu Tyr Pro Ile Phe Thr Ala Thr Gly Glu Ala Gln
             100           105           110
Arg Lys Ala Val His Glu Ala Gln Gln Cys Leu Lys Thr Leu Glu Thr
             115           120           125
Ala Leu Asp Gly Lys Lys Phe Phe Gly Gly Asp Ala Val Gly Tyr Leu
             130           135           140
Asp Ile Val Val Gly Trp Phe Ala His Trp Leu Pro Val Ile Glu Glu
             145           150           155           160
Val Thr Gly Ala Ser Val Val Thr Asp Glu Glu Leu Pro Leu Met Lys
             165           170           175
Ala Trp Phe Gly Arg Phe Leu Ala Val Asp Val Val Lys Ala Ala Leu
             180           185           190
Pro Asp Arg Asp Arg Leu Leu Ala Ala Asn Lys Ala Arg Arg Glu Gln
             195           200           205
Leu Leu Ser Ala
             210

```

```

<210> SEQ ID NO 15
<211> LENGTH: 861
<212> TYPE: DNA
<213> ORGANISM: maize

```

```

<400> SEQUENCE: 15

```

```

cggaggcgca gagcttcgac gcgcccagcg ccgagatggt ctacagcctc gccttctgc 60
cgcccaccct gcccaagcag aacgacaacg gcaacggcgg cgcgttcaac gccagggacg 120
ccaccgtagg cagcaacgcc gacgcgtcca gcggcaagcg cgggtgtggcc gggtcacagc 180
cggcggcgag ccagaccaag gtgagcgcgc agaaggagga ggagatgctg aagctgttcg 240
agcagaggaa gaaggacctg gagaagctgc tggacatcta cgagcagcgc ctggaggagg 300
ccacgttctt gcccgcgac aacttcacca togccgacct gtcgcacctg ccctacgagg 360

```



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```

accacctcgt ctccgacccg cgctcccgcc gcatgttcga gtcccgaag aacgtcagca 420
gggtggtggca cgacgtctcc ggccgcgaca cctggaagta cgtcaagacc ctgcagcgcc 480
cgccgtccac gtccaccgac gccagcgcca agaacggcca gctgggcccag cagcagcacc 540
tgccgtcgtc caccgacggc cacggcgtga agaccaacg gctggtccag aacgagcggc 600
acttctagct gttgccgtcc cttcccgccg acgaataaac tacctgcgcc gccgccaccg 660
ccgccatcca tcaacatggt tccttgtgct gttcgtgtcg ttttcatacg tcatacgtgt 720
cttgctgctt ttgaagctcc gttcccgggt gcagggacct acgagtccat tccgtcgttt 780
gctgattctg ttcgtcgtgt aataaaatga aaaccccacc ccgttttgaa tgaaaaaaaa 840
aaaaaaaaa aaaaaaaaaa a 861

```

```

<210> SEQ ID NO 16
<211> LENGTH: 190
<212> TYPE: PRT
<213> ORGANISM: maize

```

```

<400> SEQUENCE: 16

```

```

Met Val Tyr Ser Leu Ala Phe Leu Pro Pro Thr Leu Pro Lys Gln Asn
 1           5           10          15
Asp Asn Gly Asn Gly Gly Ala Phe Asn Ala Arg Asp Ala Thr Val Gly
          20           25           30
Ser Asn Ala Asp Ala Ser Ser Gly Lys Arg Gly Val Ala Gly Ser Gln
          35           40           45
Pro Ala Ala Ser Gln Thr Lys Val Ser Ala Gln Lys Glu Glu Glu Met
          50           55           60
Leu Lys Leu Phe Glu Gln Arg Lys Lys Asp Leu Glu Lys Leu Leu Asp
          65           70           75           80
Ile Tyr Glu Gln Arg Leu Glu Glu Ala Thr Phe Leu Ala Gly Asp Asn
          85           90           95
Phe Thr Ile Ala Asp Leu Ser His Leu Pro Tyr Ala Asp His Leu Val
          100          105          110
Ser Asp Pro Arg Ser Arg Arg Met Phe Glu Ser Arg Lys Asn Val Ser
          115          120          125
Arg Trp Trp His Asp Val Ser Gly Arg Asp Thr Trp Lys Tyr Val Lys
          130          135          140
Thr Leu Gln Arg Pro Pro Ser Thr Ser Thr Asp Ala Ser Ala Lys Asn
          145          150          155          160
Gly Gln Leu Gly Gln Gln Gln His Leu Pro Ser Ser Thr Asp Gly His
          165          170          175
Gly Val Lys Thr Gln Arg Leu Val Gln Asn Glu Arg His Phe
          180          185          190

```

```

<210> SEQ ID NO 17
<211> LENGTH: 917
<212> TYPE: DNA
<213> ORGANISM: maize

```

```

<400> SEQUENCE: 17

```

```

atggcggagg tggaggcgac ggtggggcga ctgatgctgt actcgtactg gcgcagctcg 60
tgctcccacc gtgcccgcac cgctctcaat ctcaaagggtg tggattacga gtacaaggcg 120
gtgaaccttc tcaagggcga gcagtctgat ccagaattcg tcaagcttaa tcctatgaag 180
ttcgtccctg cgttggttga tggcagttct gtaatagggtg actcttacgc gataaactg 240
tatttgaggg acaagtaccc agagcctcct cttctacctc aagaccttca aaagaaagct 300

```



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```

ttgaatcacc agattgcaag cattgtagct tctgggtattc aacctctcca taacctcaca 360
gtgttgaggt tcattgacca gaaggttggt gcaggggaga gtgtggttg gactcaacaa 420
caaatcgaga gaggtttcac agctattgag aacctgatac aactaaaagg atgcgccggg 480
aagtatgcaa caggagatga agtccaactg gcagatgtat tccttgacc ccagatctat 540
gcagccattg aacgcactaa aattgacatg tcaaactacc tcaactctgc taggctccac 600
tcggagtaca tgtcacaccc tgcgtttgaa gcagcgctcc ctggcaagca accggacgcc 660
ccttcaccc cctaggaact gcaccctagt gtgttggtcc tctgaatata tatatatata 720
tatgtatact tctgtaagaa ttaataatta cagagtttcg tctgctatgt cgaaaaatgt 780
caaaagtfff tgtgatttca gagactagcg gcatgaagcg tcgttgtgga tctggccgctc 840
gtcctcatgt ggcctctgtg atttcagggc atgcacttcg tcttagaagg gaaaaaaaaa 900
aaaaaaaaaa aaaaaaa 917

```

```

<210> SEQ ID NO 18
<211> LENGTH: 224
<212> TYPE: PRT
<213> ORGANISM: maize

```

```

<400> SEQUENCE: 18

```

```

Met Ala Glu Val Glu Ala Thr Val Gly Arg Leu Met Leu Tyr Ser Tyr
  1          5          10          15
Trp Arg Ser Ser Cys Ser His Arg Ala Arg Ile Ala Leu Asn Leu Lys
  20          25          30
Gly Val Asp Tyr Glu Tyr Lys Ala Val Asn Leu Leu Lys Gly Glu Gln
  35          40          45
Ser Asp Pro Glu Phe Val Lys Leu Asn Pro Met Lys Phe Val Pro Ala
  50          55          60
Leu Val Asp Gly Ser Ser Val Ile Gly Asp Ser Tyr Ala Ile Thr Leu
  65          70          75          80
Tyr Leu Glu Asp Lys Tyr Pro Glu Pro Pro Leu Leu Pro Gln Asp Leu
  85          90          95
Gln Lys Lys Ala Leu Asn His Gln Ile Ala Ser Ile Val Ala Ser Gly
 100          105          110
Ile Gln Pro Leu His Asn Leu Thr Val Leu Arg Phe Ile Asp Gln Lys
 115          120          125
Val Gly Ala Gly Glu Ser Val Leu Trp Thr Gln Gln Gln Ile Glu Arg
 130          135          140
Gly Phe Thr Ala Ile Glu Asn Leu Ile Gln Leu Lys Gly Cys Ala Gly
 145          150          155          160
Lys Tyr Ala Thr Gly Asp Glu Val Gln Leu Ala Asp Val Phe Leu Ala
 165          170          175
Pro Gln Ile Tyr Ala Ala Ile Glu Arg Thr Lys Ile Asp Met Ser Asn
 180          185          190
Tyr Leu Thr Leu Ala Arg Leu His Ser Glu Tyr Met Ser His Pro Ala
 195          200          205
Phe Glu Ala Ala Leu Pro Gly Lys Gln Pro Asp Ala Pro Ser Ser Ser
 210          215          220

```

```

<210> SEQ ID NO 19
<211> LENGTH: 919
<212> TYPE: DNA
<213> ORGANISM: maize

```



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&lt;400&gt; SEQUENCE: 19

```

cacctgctgt atctcattac catctgcatc tggttgcccg ttgattgaga aggaggagct    60
gagggccatg gcgaccgaga agcccacccct gtacaacgcc tggatcagct cctgctccca    120
ccgtgttcgc atcgactca acctcaaagg tgtggattac gagtacaagt cggtaaacc    180
taggacagat ccagattatg aaaaaatcaa tccaatcaaa tatattccag cattagtaga    240
tggggacata gtcgtttctg attctcttgc catctcattg tatttggaag ataagtatcc    300
tgagcatcca ctctgccta aagatctcaa gaggaaagct cttaatcttc agattgcaaa    360
cattgtttgt tcaagcattc aacctcttca aggctatgct gttattggtc tgcacgaggg    420
taggatgagc ccagatgagg gccttcatat tgttcaaagt tatattgaca aggattcag    480
agcgatcgaa aagctgttgg aaggatgtga gagtaaatat gctactggag atgatgtcca    540
attggcagat gtgttccttg aaccacagat acatgccggc ataaatcgct tccaaatcga    600
tatgtcgatg tacccaatct tggagaggct ccatgatgca tacatgcaaa ttcccgcatt    660
ccaagccgcg cttcctaaaa atcaaccaga cgcaccttca tcataatcat caagattatc    720
tcaataattt gcatgtcatt ttgtaataat ttggataggg agccactgct tcctccatcc    780
cgttgtggat caaaaggtg aacgattggc acttacctgc atggtccaat acctattata    840
tttcttaaac agatactatt tacggctatt gtaatttaag cccaaaaaaa aaaaaaaaaa    900
aaaaaaaaaa aaaaaaaaaa                                         919

```

&lt;210&gt; SEQ ID NO 20

&lt;211&gt; LENGTH: 212

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: maize

&lt;400&gt; SEQUENCE: 20

```

Met Ala Thr Glu Lys Pro Ile Leu Tyr Asn Ala Trp Ile Ser Ser Cys
  1           5           10          15
Ser His Arg Val Arg Ile Ala Leu Asn Leu Lys Gly Val Asp Tyr Glu
  20          25          30
Tyr Lys Ser Val Asn Pro Arg Thr Asp Pro Asp Tyr Glu Lys Ile Asn
  35          40          45
Pro Ile Lys Tyr Ile Pro Ala Leu Val Asp Gly Asp Ile Val Val Ser
  50          55          60
Asp Ser Leu Ala Ile Ser Leu Tyr Leu Glu Asp Lys Tyr Pro Glu His
  65          70          75          80
Pro Leu Leu Pro Lys Asp Leu Lys Arg Lys Ala Leu Asn Leu Gln Ile
  85          90          95
Ala Asn Ile Val Cys Ser Ser Ile Gln Pro Leu Gln Gly Tyr Ala Val
 100          105          110
Ile Gly Leu His Glu Gly Arg Met Ser Pro Asp Glu Gly Leu His Ile
 115          120          125
Val Gln Ser Tyr Ile Asp Lys Gly Phe Arg Ala Ile Glu Lys Leu Leu
 130          135          140
Glu Gly Cys Glu Ser Lys Tyr Ala Thr Gly Asp Asp Val Gln Leu Ala
 145          150          155          160
Asp Val Phe Leu Glu Pro Gln Ile His Ala Gly Ile Asn Arg Phe Gln
 165          170          175
Ile Asp Met Ser Met Tyr Pro Ile Leu Glu Arg Leu His Asp Ala Tyr
 180          185          190
Met Gln Ile Pro Ala Phe Gln Ala Ala Leu Pro Lys Asn Gln Pro Asp

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195	200	205	
Ala Pro Ser Ser			
210			
<210> SEQ ID NO 21			
<211> LENGTH: 996			
<212> TYPE: DNA			
<213> ORGANISM: maize			
<400> SEQUENCE: 21			
catcgatccg ccattgctca cgcacaagt gcacgctcac ctcacacacg cagctaagta			60
gctaacgccg taggcgagaa caagaaaagg ctcgacatgg ccgaggagaa gaagcagggc			120
ctgcagctgc tggacttctg ggtgagccca ttcgggcagc gctgccgcat cgcgctggac			180
gagaagggcc tggcctacga gtacctggag caggacctga ggaacaagag cgagctgctc			240
ctccgcgcca acccggtgca caagaagatc cccgtgctgc tgcacgacgg ccgccccgtc			300
tgcgagtccc tcgtcatcgt gcagtacctc gacgaggcgt tcccggaggc ggcgccggcg			360
ctgctccccg ccgacccta cgcgcgcgcg caggcccgtc tctgggcgga ctacgtcgac			420
aagaagctgt acgactgcgg caccggctg tggaaagctca agggggacgg ccaggcgag			480
gcgcgcgccg agatggtcga gatcctccgc acgctggagg gcgcgctcgg cgacgggccc			540
ttcttcggtg gcgacgccct cggcttcgtc gacgtcgcgc tcgtgccctt cacgtcctgg			600
ttcctcgcct acgaccgctt cggcggcgtc agcgtggaga aggagtgccg gaggtggcc			660
gcctgggcca agcgtcgcgc cgagcgcccc agcgtcgcca agaacctcta cccgcccag			720
aaggtctacg acttcgtctg cgggatgaag aagaggctgg gcatcgagta gagcatccat			780
cggtcggccg gtggctggcc gggagtaata atgacgaacc aataatctag ttttggtttt			840
agtgtgctca gcagagcagt tcgtgttcat gagttcgtcg tcgttgattt ttctattgtc			900
agcgggtgca gcgccgtacg tgttgctcgc tacaccacaa ccgaataaat ggttatgaat			960
ttcttcttgt tgtcttaaaa aaaaaaaaaa aaaaaa			996

<210> SEQ ID NO 22  
 <211> LENGTH: 224  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 22

Met Ala Glu Glu Lys Lys Gln Gly Leu Gln Leu Leu Asp Phe Trp Val															
1				5				10							15
Ser Pro Phe Gly Gln Arg Cys Arg Ile Ala Leu Asp Glu Lys Gly Leu															
			20					25						30	
Ala Tyr Glu Tyr Leu Glu Gln Asp Leu Arg Asn Lys Ser Glu Leu Leu															
			35					40						45	
Leu Arg Ala Asn Pro Val His Lys Lys Ile Pro Val Leu Leu His Asp															
			50					55						60	
Gly Arg Pro Val Cys Glu Ser Leu Val Ile Val Gln Tyr Leu Asp Glu															
			65					70						75	80
Ala Phe Pro Glu Ala Ala Pro Ala Leu Leu Pro Ala Asp Pro Tyr Ala															
			85					90						95	
Arg Ala Gln Ala Arg Phe Trp Ala Asp Tyr Val Asp Lys Lys Leu Tyr															
			100					105						110	
Asp Cys Gly Thr Arg Leu Trp Lys Leu Lys Gly Asp Gly Gln Ala Gln															
			115					120						125	

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Ala Arg Ala Glu Met Val Glu Ile Leu Arg Thr Leu Glu Gly Ala Leu  
 130 135 140

Gly Asp Gly Pro Phe Phe Gly Gly Asp Ala Leu Gly Phe Val Asp Val  
 145 150 155 160

Ala Leu Val Pro Phe Thr Ser Trp Phe Leu Ala Tyr Asp Arg Phe Gly  
 165 170 175

Gly Val Ser Val Glu Lys Glu Cys Pro Arg Leu Ala Ala Trp Ala Lys  
 180 185 190

Arg Cys Ala Glu Arg Pro Ser Val Ala Lys Asn Leu Tyr Pro Pro Glu  
 195 200 205

Lys Val Tyr Asp Phe Val Cys Gly Met Lys Lys Arg Leu Gly Ile Glu  
 210 215 220

<210> SEQ ID NO 23  
 <211> LENGTH: 895  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 23

```

ggcacgagac gacatcgaag gagcctgcga agcgagcgag agtctataat ggcggacgga      60
ggcgagctgc agctgctggg ctcatggtac agcccctacg tgatccgcgc caaggtggcg      120
ctggggctga aggggctcag ctacgagttc gtcgaggagg acctctcccg caagagcgac      180
ctgctgctga agctcaaccc ggtgcacagg aaggtgcccg tgctggtcca cggcggcccg      240
cccgtgtgcg agtcgctcgt catcctgcag tacgtcgacg agacctgggc aggcaccggg      300
accctctcc tccccgccga cgcctacgac cgcgccatgg ctgcttctg ggcagcctac      360
gtcgacgaca agttctacaa ggagtggaac cggctgttct ggtcgacgac ggcggagaag      420
gcgggcgagg cgctcggcgt cgctcgtccc gtggtggaga cgctggagca ggcgttcagg      480
gagtgtctca aagggaaacc ttcttcggcg gcgacgccgt cgggctcgtg gacatcgcgc      540
tcgggagctt cgtggtgtgg atcagggagg tggacgaggg ggcggcgta aagcttctgg      600
acgaggccaa gttcccggcc ttgacggcgt gggcggagcg cttcttggcg gtggacgccg      660
tgaaggaggt gatgccggac gccggaaggc tggtggagca ctacaagggg tttctggcta      720
aacggtctcc acctgctggt tactgaacgc tgtaactgta agcctgtaac agcaagctca      780
gtgttcgtgt acttttccgt gcgttaacgt gtactagagt tcaggaaagg ctttgattct      840
gtccagagtc cagacgaata aacgaatggt ttttataaaa aaaaaaaaaa aaaaaa      895

```

<210> SEQ ID NO 24  
 <211> LENGTH: 180  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 24

Met Ala Asp Gly Gly Glu Leu Gln Leu Leu Gly Ser Trp Tyr Ser Pro  
 1 5 10 15

Tyr Val Ile Arg Ala Lys Val Ala Leu Gly Leu Lys Gly Leu Ser Tyr  
 20 25 30

Glu Phe Val Glu Glu Asp Leu Ser Arg Lys Ser Asp Leu Leu Leu Lys  
 35 40 45

Leu Asn Pro Val His Arg Lys Val Pro Val Leu Val His Gly Gly Arg  
 50 55 60

Pro Val Cys Glu Ser Leu Val Ile Leu Gln Tyr Val Asp Glu Thr Trp  
 65 70 75 80



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Ala Gly Thr Gly Thr Pro Leu Leu Pro Ala Asp Ala Tyr Asp Arg Ala  
                                   85                                  90                                  95

Met Ala Arg Phe Trp Ala Ala Tyr Val Asp Asp Lys Phe Tyr Lys Glu  
                                   100                                  105                                  110

Trp Asn Arg Leu Phe Trp Ser Thr Thr Ala Glu Lys Ala Ala Glu Ala  
                                   115                                  120                                  125

Leu Gly Val Val Val Pro Val Val Glu Thr Leu Glu Gln Ala Phe Arg  
                                   130                                  135                                  140

Glu Cys Ser Lys Gly Lys Pro Ser Ser Ala Ala Thr Pro Ser Gly Ser  
                                   145                                  150                                  155                                  160

Trp Thr Ser Arg Ser Gly Ala Ser Trp Cys Gly Ser Gly Trp Trp Thr  
                                   165                                  170                                  175

Arg Arg Pro Ala  
                                   180

<210> SEQ ID NO 25  
 <211> LENGTH: 1279  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 25

```

ctgcaggttc agttcagtag tgtgctctga cagtgagatg gcgagcgtga aggttttcgg      60
gtcaccacc  tcggcggagg tcgcccgcgt gctcatgtgc ctcttcgaga aggaggtgga      120
gttcacgctg atccgcgtcg acgcctaccg cggcaccaag cgcattgccc agtacctcaa      180
gctgcagccg caaggcgagg cgctcacctt cgaggacgag agcctcacc  tctccgactc      240
cagggggatc ctccgccaca tctcccacaa gtacgcgaag cagggcaacc cgttacctga      300
ttggcacggg cgcgctggag cgggcgtcca tcgagcagtg gctgcagacg gaggcgcaga      360
gcttcgacgc gccagcgcc  gagatggtct acagcctcgc cttcctgccg cccaccctgc      420
ccaagcagaa cgacaacggc aacggcggcg cgttcaacgc cagggacgcc accgtaggca      480
gcaacgccga cgcgtccagc ggcaagcgcg gtgtggccgg gtcacagccg gcggcgagcc      540
agaccaaggt gagcgcgcag aaggaggagg agatgctgaa gctgttcgag cagaggaaga      600
aggacctgga gaagctgctg gacatctacg agcagcgcct ggaggaggcc acgttcctgg      660
ccggcgacaa cttcaccatc gccgacctgt cgcacctgcc ctacgcggac cacctcgtct      720
ccgaccgcg  ctcccgcgc  atgttcgagt cccgcaagaa cgtcagcagg tgggtggcacg      780
acgtctccgg ccgcgacacc tggaagtacg tcaagaccct gcagcgcgcc cgtccacgt      840
ccaccgacgc cagcgcgaag aacggccagc tgggcccagca gcagcacctg ccgtcgtcca      900
ccgacggcca cggcgtgaag acccaacggc tgggtccagaa cgagcggcac ttctagctgt      960
tgccgtccct tcccgcggac gaataaacta cctgcgcgcg cgccaccgcc gccatccatc     1020
aacatggttc cttgtgctgt tcgtgtcgtt ttcatacgtc atacgtgtct tgctgctttt     1080
gaagctccgt tcccgggtgc agggacctac gagtccattc cgtcgtttgc tgattctgtt     1140
cgtcgtgtaa taaaatgaaa accccacccc gttttgaatg aaattcaatt ctcagtgtcg     1200
tgtgaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa     1260
aaaaaaaaaa aaaaaaaaaa

```

<210> SEQ ID NO 26  
 <211> LENGTH: 370  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

-continued

&lt;400&gt; SEQUENCE: 26

Met Ala Ser Val Lys Val Phe Gly Ser Pro Thr Ser Ala Glu Val Ala  
 1 5 10 15  
 Arg Val Leu Met Cys Leu Phe Glu Lys Glu Val Glu Phe Gln Leu Ile  
 20 25 30  
 Arg Val Asp Ala Tyr Arg Gly Thr Lys Arg Met Pro Gln Tyr Leu Lys  
 35 40 45  
 Leu Gln Pro Gln Gly Glu Ala Leu Thr Phe Glu Asp Glu Ser Leu Thr  
 50 55 60  
 Leu Ser Asp Ser Arg Gly Ile Leu Arg His Ile Ser His Lys Tyr Ala  
 65 70 75 80  
 Lys Gln Gly Asn Pro Leu Pro Asp Trp His Gly Arg Ala Gly Ala Gly  
 85 90 95  
 Val His Arg Ala Val Ala Ala Asp Gly Gly Ala Glu Leu Arg Arg Ala  
 100 105 110  
 Gln Arg Arg Asp Gly Leu Gln Pro Arg Leu Pro Ala Ala His Pro Ala  
 115 120 125  
 Gln Ala Glu Arg Gln Arg Gln Arg Arg Arg Val Gln Arg Gln Gly Arg  
 130 135 140  
 His Arg Arg Gln Gln Arg Arg Arg Val Gln Arg Gln Ala Arg Cys Gly  
 145 150 155 160  
 Arg Val Thr Ala Gly Gly Glu Pro Asp Gln Gly Glu Arg Ala Glu Gly  
 165 170 175  
 Gly Gly Asp Ala Glu Ala Val Arg Ala Glu Glu Glu Gly Pro Gly Glu  
 180 185 190  
 Ala Ala Gly His Leu Arg Ala Ala Pro Gly Gly Gly His Val Pro Gly  
 195 200 205  
 Arg Arg Gln Leu His His Arg Arg Pro Val Ala Pro Ala Leu Arg Gly  
 210 215 220  
 Pro Pro Arg Leu Arg Pro Ala Leu Pro Pro His Val Arg Val Pro Gln  
 225 230 235 240  
 Glu Arg Gln Gln Val Val Ala Arg Arg Leu Arg Pro Arg His Leu Glu  
 245 250 255  
 Val Arg Gln Asp Pro Ala Ala Pro Ala Val His Val His Arg Arg Gln  
 260 265 270  
 Arg Gln Glu Arg Pro Ala Gly Pro Ala Ala Ala Pro Ala Val Val His  
 275 280 285  
 Arg Arg Pro Arg Arg Glu Asp Pro Thr Ala Gly Pro Glu Arg Ala Ala  
 290 295 300  
 Leu Leu Ala Val Ala Val Pro Ser Arg Arg Arg Ile Asn Tyr Leu Arg  
 305 310 315 320  
 Arg Arg His Arg Arg His Pro Ser Thr Trp Phe Leu Val Leu Phe Val  
 325 330 335  
 Ser Phe Ser Tyr Val Ile Arg Val Leu Leu Leu Lys Leu Arg Ser  
 340 345 350  
 Arg Val Gln Gly Pro Thr Ser Pro Phe Arg Arg Leu Leu Ile Leu Phe  
 355 360 365  
 Val Val  
 370

&lt;210&gt; SEQ ID NO 27

&lt;211&gt; LENGTH: 1198

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: maize



-continued

&lt;400&gt; SEQUENCE: 27

```

ctggctcacc tcacctgcag caggcctcgt ctcggtcac cagcgcattg ctctcaatcg      60
ctgcagcgca tccagtccaa acacacaccg gtcgaatcga gcaatggccg cggggctgca     120
ggtgttcggg cagccggctt ccaccgacgt cgcaggggtg ctgacctgcc tcttcgagaa     180
gaacctcgag ttcgagctcg tccgcaccga caccttcaag aagtcgcaca agctccccga     240
gttcatcaag ctgagggatc ctaccgggca ggtgactttc aagcacgggtg acaagacaat     300
cgttgattcc aggactatct gccggtacct gtgcacgcag ttcccggacg acgggtacaa     360
gaagctgtac ggcacggggt cgctggagcg ggcgtccata gagcagtggc tgcaggcgga     420
ggcgagagc ttcgacgcgc cgagctcggg gctggcggtt cagctggcgt tcgcgccgca     480
cctcaaggac gtgcggcccg acgaggcccg cgtcgcggag aacgagaaga agctgcacag     540
catgctgggc gtctacgacg acatcctctc caagaacgag tacctcgccg gcgacgactt     600
cacactggcc gacctctccc acctgccaaa ctcccactac atcgtcaact cctccgacag     660
gggcaggaag ctcttcaccg ccaggaagca cgtggccagg tggtagaca agatctccac     720
ccgcgactcc tggaggcagg tcatgaagat gcagagggag caccgccggc cgttcgagtg     780
atgcgtcgtg cttccttctc tctgcatgca tgcgcgcgtc gcggcgtggt cctcgtcgtc     840
gccggcttcg tggtcgtcag gcttcacacc gtgggtgtgtg gttgtcgagt tcgtcgtatt     900
tcgtatcgta tcgtatcgta tcgtacgtac gtctctgtggg ctaaataaac gtggagcctg     960
cgcctgccta cgggtgtctc cgtgtcttcc tttcgtcgtc tatataagca gtgtcttttt    1020
ctgggtattg tatggtgacc taatcatcaa ctctctttgc aatattggcc aattcaataa    1080
aaatatgtcc gggcattttg ctcgttcgca ctaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    1140
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    1198

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&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 225

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: maize

&lt;400&gt; SEQUENCE: 28

```

Met Ala Ala Gly Leu Gln Val Phe Gly Gln Pro Ala Ser Thr Asp Val
  1           5           10          15
Ala Arg Val Leu Thr Cys Leu Phe Glu Lys Asn Leu Glu Phe Glu Leu
  20          25          30
Val Arg Thr Asp Thr Phe Lys Lys Ser His Lys Leu Pro Glu Phe Ile
  35          40          45
Lys Leu Arg Asp Pro Thr Gly Gln Val Thr Phe Lys His Gly Asp Lys
  50          55          60
Thr Ile Val Asp Ser Arg Thr Ile Cys Arg Tyr Leu Cys Thr Gln Phe
  65          70          75          80
Pro Asp Asp Gly Tyr Lys Lys Leu Tyr Gly Thr Gly Ser Leu Glu Arg
  85          90          95
Ala Ser Ile Glu Gln Trp Leu Gln Ala Glu Ala Gln Ser Phe Asp Ala
 100         105         110
Pro Ser Ser Glu Leu Ala Phe Gln Leu Ala Phe Ala Pro His Leu Lys
 115         120         125
Asp Val Arg Pro Asp Glu Ala Arg Val Ala Glu Asn Glu Lys Lys Leu
 130         135         140
His Ser Met Leu Gly Val Tyr Asp Asp Ile Leu Ser Lys Asn Glu Tyr

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145	150	155	160
Leu Ala Gly Asp Asp Phe Thr Leu Ala Asp Leu Ser His Leu Pro Asn	165	170	175
Ser His Tyr Ile Val Asn Ser Ser Asp Arg Gly Arg Lys Leu Phe Thr	180	185	190
Ala Arg Lys His Val Ala Arg Trp Tyr Asp Lys Ile Ser Thr Arg Asp	195	200	205
Ser Trp Arg Gln Val Met Lys Met Gln Arg Glu His Pro Gly Ala Phe	210	215	220
Glu			
225			

<210> SEQ ID NO 29  
 <211> LENGTH: 1134  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 29

```

tcatcatcca ggcgccgag tntaggtcta gatcatccaa tccaacacac cggtcgagca    60
atggcggcag ggctgcaggt gttcggacag cggcgtcca cgcacgtcgc gagggtgctg    120
acctgcctct tcgagaagaa cctcgagttc gagctcatcc gcaccgacac cttcaagaag    180
tcccacaagc tccccgagtt catcaagcta agggatccta ctgggcaggt gactttcaag    240
cacggtgaca aaacaatcgt tgattccagg gccatttgcc ggtacctgtg cacgcagttc    300
ccggacgacg ggtacaagaa gctgtacggg acgggggtcgc tggagcgggc gtccatagag    360
cagtggctgc aggcggaggc ccagagcttc gacgcgccga gctcggagct ggcgttccag    420
ctggcgttcg cgccgcacct caagaacgtg cggcccgcgc aggccgcgc cgcggagaac    480
gagaggaagc tgcacggcat gctgggcgtc tacgacgaca tcctctcaa gaacgagtac    540
ctgcgccgag acgacttcac cctggccgac ctctccacc tgcccaactc cactacatc    600
gtcaactcct ccgacagggg cagaaagctc ttcaccgcca ggaagcacgt cgcaggtgg    660
tacgacaaga tctccaccg cgactcgtgg aggcaggtca tcaagatgca gagggagcac    720
cccggcgcgt tcgagtgatc ggtcgggtgt ctacgcggtg atgcatgcat gcatgcatgc    780
gccgcggcgt gttcctcgat cgccgccagc caccggcggc ttcgtcgtcg tcaggcttcg    840
taccttgacg gggttgtcga cttcgtcgtg cgtccctgtg gcctgtgggc taaaataacg    900
tgaagcctgc ctacgcggtg tctcgtgtct taccttttaa atttgcacca tatatacgca    960
ctgtcttttc tgggtatttg ttgtattgtg atgtacggag tattcatcaa ctctttttgc  1020
aagattggtc aattattcag gcaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa  1080
aaaaaaaaaa agaaaaaaaa aaaanaaaaa aaaaaaaaaa aaaaaaaaaa aana      1134

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<210> SEQ ID NO 30  
 <211> LENGTH: 225  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 30

Met Ala Ala Gly Leu Gln Val Phe Gly Gln Pro Ala Ser Thr Asp Val	1	5	10	15
Ala Arg Val Leu Thr Cys Leu Phe Glu Lys Asn Leu Glu Phe Glu Leu	20	25	30	
Ile Arg Thr Asp Thr Phe Lys Lys Ser His Lys Leu Pro Glu Phe Ile	35	40	45	



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Lys Leu Arg Asp Pro Thr Gly Gln Val Thr Phe Lys His Gly Asp Lys  
     50                                    55                                    60  
 Thr Ile Val Asp Ser Arg Ala Ile Cys Arg Tyr Leu Cys Thr Gln Phe  
     65                                    70                                    75                                    80  
 Pro Asp Asp Gly Tyr Lys Lys Leu Tyr Gly Thr Gly Ser Leu Glu Arg  
                                     85                                    90                                    95  
 Ala Ser Ile Glu Gln Trp Leu Gln Ala Glu Ala Gln Ser Phe Asp Ala  
                                     100                                    105                                    110  
 Pro Ser Ser Glu Leu Ala Phe Gln Leu Ala Phe Ala Pro His Leu Lys  
                                     115                                    120                                    125  
 Asn Val Arg Pro Asp Glu Ala Arg Ala Ala Glu Asn Glu Arg Lys Leu  
     130                                    135                                    140  
 His Gly Met Leu Gly Val Tyr Asp Asp Ile Leu Ser Lys Asn Glu Tyr  
     145                                    150                                    155                                    160  
 Leu Ala Gly Asp Asp Phe Thr Leu Ala Asp Leu Ser His Leu Pro Asn  
                                     165                                    170                                    175  
 Ser His Tyr Ile Val Asn Ser Ser Asp Arg Gly Arg Lys Leu Phe Thr  
                                     180                                    185                                    190  
 Ala Arg Lys His Val Ala Arg Trp Tyr Asp Lys Ile Ser Thr Arg Asp  
                                     195                                    200                                    205  
 Ser Trp Arg Gln Val Ile Lys Met Gln Arg Glu His Pro Gly Ala Phe  
     210                                    215                                    220

Glu  
225

<210> SEQ ID NO 31  
 <211> LENGTH: 1007  
 <212> TYPE: DNA  
 <213> ORGANISM: maize  
 <400> SEQUENCE: 31

aacacaggct gttgtttgc tcttttggt aaggctttag ctgcggcaga tccaccggcg 60  
 gcgccccgag aatcgaagat gccggtgaag gtgttcggat cgccgacgtc ggcggaggtc 120  
 gcccgcgtcc tggcctgcct gttcgagaag gacgtcgagt tccagctcat ccgcgtcgac 180  
 tccttccgcg gcaccaagcg cctgccccag tacctcaagc tccagccgca cggcgaggcg 240  
 ctcaccttcg aggacggcaa cgtcaccctc gtcgagtcga ggaagatcct gcgccacatc 300  
 gccgacaagt acaagaacca ggggtacagg gacctgttcg gcccgggcgc gctggagcgg 360  
 gcctccatcg agcagtggct gcagacggag gcgcagagct tcgacgtccc cagcgccgac 420  
 atggtctaca gcctcgccta cctgccgccc gacatgcagc tcgacggcag gggcgtcggc 480  
 ggcctcccgg cggcgacggg gacgatgaac ccggcgcacc ggcagaaggt ggaggagatg 540  
 ctgcagctgt tcgagaagag ccgcaggcag ctgggcaagc tgctggacat ctacgagcag 600  
 cgccttggcg aggagcctt cctggccgga ggcaagttca cgctcgcga cctgtcccac 660  
 ctgcccaacg ccgaccgct cgccggcgac ccgcggtccg cagcctcat ggagtgcgcg 720  
 aggaacgtca gcaagtgggt ggacaccgta tcccgcgcg actcttgggt cagggtcaag 780  
 gagttgcagc gcccgccgtc cgcggaggcg cccttctgat gtcgatcgat cgcaaattaa 840  
 ggcggtggcc tttgctcaag cctacgtggt cggtttctgc ataatttttt aataaataaa 900  
 cgccactggc ccctctacgt cattggcgat tgttcattgt gtaataaatc gttcaagagc 960  
 atatgatgct tcttgccgtg aaaaaaaaa aaaaaaaaa aaaaaaa 1007

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<210> SEQ ID NO 32  
 <211> LENGTH: 246  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 32

Met Pro Val Lys Val Phe Gly Ser Pro Thr Ser Ala Glu Val Ala Arg  
 1 5 10 15  
 Val Leu Ala Cys Leu Phe Glu Lys Asp Val Glu Phe Gln Leu Ile Arg  
 20 25 30  
 Val Asp Ser Phe Arg Gly Thr Lys Arg Leu Pro Gln Tyr Leu Lys Leu  
 35 40 45  
 Gln Pro His Gly Glu Ala Leu Thr Phe Glu Asp Gly Asn Val Thr Leu  
 50 55 60  
 Val Glu Ser Arg Lys Ile Leu Arg His Ile Ala Asp Lys Tyr Lys Asn  
 65 70 75 80  
 Gln Gly Tyr Arg Asp Leu Phe Gly Pro Gly Ala Leu Glu Arg Ala Ser  
 85 90 95  
 Ile Glu Gln Trp Leu Gln Thr Glu Ala Gln Ser Phe Asp Val Pro Ser  
 100 105 110  
 Ala Asp Met Val Tyr Ser Leu Ala Tyr Leu Pro Pro Asp Met Gln Leu  
 115 120 125  
 Asp Gly Arg Gly Val Gly Gly Leu Pro Ala Ala Thr Gly Thr Met Asn  
 130 135 140  
 Pro Ala His Arg Gln Lys Val Glu Glu Met Leu Gln Leu Phe Glu Lys  
 145 150 155 160  
 Ser Arg Arg Gln Leu Gly Lys Leu Leu Asp Ile Tyr Glu Gln Arg Leu  
 165 170 175  
 Gly Glu Glu Ala Phe Leu Ala Gly Gly Lys Phe Thr Leu Ala Asp Leu  
 180 185 190  
 Ser His Leu Pro Asn Ala Asp Arg Leu Ala Gly Asp Pro Arg Ser Ala  
 195 200 205  
 Arg Leu Met Glu Ser Arg Arg Asn Val Ser Lys Trp Trp Asp Thr Val  
 210 215 220  
 Ser Arg Arg Asp Ser Trp Val Arg Val Lys Glu Leu Gln Arg Pro Pro  
 225 230 235 240  
 Ser Ala Glu Ala Pro Phe  
 245

<210> SEQ ID NO 33  
 <211> LENGTH: 911  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 33

cggagcaaga gaaagccat ggctacgccg gcggcggtga tgaagttgta cgggtgggct 60  
 atctcgccgt tcgtgtcgcg ggctctgctg gccctggagg aggccggcgt cgactacgag 120  
 ctctgccccca tgagccccca ggccggcgac caccggcgcc cggagcacct cgccaggaac 180  
 cctttcgcca tggtgccggt gctcgaggac ggcgacctca cgctctttga atcccgggcg 240  
 atcgcgaggc acgttctccg caagcacagg ccggagctcc tgggcgccgg cgccggcggc 300  
 agcctcgagc gggcgccgat ggtggacgtg tggctcgagg tggaggcgca ccagctgagc 360  
 ccgccagcgg tcgccatcgt ggtggagtgc ttcgctgcgc cgctgctcgg ccgagagcgc 420  
 gaccagacgg tcgtcgacga gaacgtggag aagctcagga aggtgctcga ggtgtacgag 480



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gcgcggttg gcgagtgcag gtacctgcc ggcgacttcc tcagcctcgc cgacctcagc 540
cccttcacca tcatgcaactg catcatggcc accgagtacg ccgccgccct ggtcgaggcg 600
ctcccgcgcg tcagcgcctg gtgggagggc ctgcgccgcg gccccgcggc caagaaggtg 660
gcgagattca taccggtcgg cgcggccgga ctgctggagc accctcccaa acaacaggat 720
tgatgcatga tgaagcaagc ctgcctaatag tgctgtttgc gcttaatact ttcccacgtg 780
tactttccca caacgttgac agaagttatt caataactag tctctatgta acgtaatggt 840
gtggtgtgca cttcaatgaa taccatgagg tggctggttc aaaaaaaaaa aaaaaaaaaa 900
aaaaaaaaaa a 911

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<210> SEQ ID NO 34
<211> LENGTH: 234
<212> TYPE: PRT
<213> ORGANISM: maize

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<400> SEQUENCE: 34

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Met Ala Thr Pro Ala Ala Val Met Lys Leu Tyr Gly Trp Ala Ile Ser
 1             5             10             15
Pro Phe Val Ser Arg Ala Leu Leu Ala Leu Glu Glu Ala Gly Val Asp
          20             25             30
Tyr Glu Leu Val Pro Met Ser Pro Gln Ala Gly Asp His Arg Arg Pro
          35             40             45
Glu His Leu Ala Arg Asn Pro Phe Ala Met Val Pro Val Leu Glu Asp
          50             55             60
Gly Asp Leu Thr Leu Phe Glu Ser Arg Ala Ile Ala Arg His Val Leu
          65             70             75             80
Arg Lys His Arg Pro Glu Leu Leu Gly Ala Gly Ala Gly Gly Ser Leu
          85             90             95
Glu Arg Ala Ala Met Val Asp Val Trp Leu Glu Val Glu Ala His Gln
          100            105            110
Leu Ser Pro Pro Ala Val Ala Ile Val Val Glu Cys Phe Ala Ala Pro
          115            120            125
Leu Leu Gly Arg Glu Arg Asp Gln Thr Val Val Asp Glu Asn Val Glu
          130            135            140
Lys Leu Arg Lys Val Leu Glu Val Tyr Glu Ala Arg Leu Gly Glu Cys
          145            150            155            160
Arg Tyr Leu Ala Gly Asp Phe Leu Ser Leu Ala Asp Leu Ser Pro Phe
          165            170            175
Thr Ile Met His Cys Ile Met Ala Thr Glu Tyr Ala Ala Ala Leu Val
          180            185            190
Glu Ala Leu Pro Arg Val Ser Ala Trp Trp Glu Gly Leu Ala Ala Arg
          195            200            205
Pro Ala Ala Lys Lys Val Ala Glu Phe Ile Pro Val Gly Ala Ala Gly
          210            215            220
Leu Leu Glu His Pro Pro Lys Gln Gln Asp
          225            230

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<210> SEQ ID NO 35
<211> LENGTH: 1098
<212> TYPE: DNA
<213> ORGANISM: maize

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<400> SEQUENCE: 35

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gcagcattgt accctatggt catgccacca tggaagggct tcgtattgga gcaccgatta 60

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tgcaggttta tcatgagaaa tcttttatct tacctgatgt ttcaaggggtg cttgcttgcc 120
tttatgagaa ggatgtcaag tttgagactc acacagcctc atacaggagc ctactcggat 180
tgcaggcatc atctcatgct ccagttccat tctatgaagg ccctactttt ctagaagaat 240
ccagagaaat ctgccgttat atagcagaaa agtatgaaaa tcaaggatat ccgttcctcc 300
ttgaaaagga tgcccttgag agggcttcaa ttgaacaatg gctccacaac gaggagcatg 360
ctttcaacc cccgagccgg gccttggtct ttcatttggc ctttcccctg ggtgaaggag 420
aagatgatga tattgatggt catacaagga agctagaaga ggttctggaa gtttatgagc 480
aaaggctcag tgacagcgaa ttccttggtg gaaacaagtt cactcttgcc gaccttgttc 540
acctgccaaa ttcccactat atcaaagcat ctaacaagtt tctttacctt tatgattcga 600
ggaaaaatgt aaggaggtgg tgggatgcta tttctgaccg gagttcttgg aagaaagtgc 660
tgaggtatat gaagagcgtg gaggagaaga acaacaaga agaactcaag aagcagcagc 720
agcagcagga agaggctcct agaacctcca ccgaccaac tcgggtagac tcgagaaagc 780
agagcagaac agagcctcgg acaatattgg ttctctctgc tgataacgag tcatcagctt 840
cgatagttcc tcgaacaaag aagcctcttc ctgggatgca cttagtgtct actcaacaaa 900
ttgatgggtg tggtatgcca gccacaaatt gatgggatg gtcgtcttag tgggttttgt 960
cttgtctttt attgtttggt tctttaacaa gagttatatt tttaccatca aaaaaaaaaa 1020
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1080
aaaaaaaaa aaaaaaaa 1098

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&lt;210&gt; SEQ ID NO 36

&lt;211&gt; LENGTH: 300

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: maize

&lt;400&gt; SEQUENCE: 36

```

Met Glu Gly Leu Arg Ile Gly Ala Pro Ile Met Gln Val Tyr His Glu
  1             5             10             15
Lys Ser Phe Ile Leu Pro Asp Val Ser Arg Val Leu Ala Cys Leu Tyr
          20             25             30
Glu Lys Asp Val Lys Phe Glu Thr His Thr Ala Ser Tyr Arg Ser Leu
          35             40             45
Leu Gly Leu Gln Ala Ser Ser His Ala Pro Val Pro Phe Tyr Glu Gly
          50             55             60
Pro Thr Phe Leu Glu Glu Ser Arg Glu Ile Cys Arg Tyr Ile Ala Glu
          65             70             75             80
Lys Tyr Glu Asn Gln Gly Tyr Pro Phe Leu Leu Gly Lys Asp Ala Leu
          85             90             95
Glu Arg Ala Ser Ile Glu Gln Trp Leu His Asn Glu Glu His Ala Phe
          100            105            110
Asn Pro Pro Ser Arg Ala Leu Phe Phe His Leu Ala Phe Pro Leu Gly
          115            120            125
Glu Gly Glu Asp Asp Asp Ile Asp Val His Thr Arg Lys Leu Glu Glu
          130            135            140
Val Leu Glu Val Tyr Glu Gln Arg Leu Ser Asp Ser Glu Phe Leu Val
          145            150            155            160
Gly Asn Lys Phe Thr Leu Ala Asp Leu Val His Leu Pro Asn Ser His
          165            170            175
Tyr Ile Lys Ala Ser Asn Lys Phe Leu Tyr Leu Tyr Asp Ser Arg Lys

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	180		185		190														
Asn	Val	Arg	Arg	Trp	Trp	Asp	Ala	Ile	Ser	Asp	Arg	Ser	Ser	Trp	Lys				
	195						200					205							
Lys	Val	Leu	Arg	Tyr	Met	Lys	Ser	Val	Glu	Glu	Lys	Asn	Lys	Gln	Glu				
	210					215					220								
Glu	Leu	Lys	Lys	Gln	Gln	Gln	Gln	Gln	Glu	Glu	Ala	Pro	Arg	Thr	Ser				
	225				230					235					240				
Thr	Asp	Pro	Thr	Arg	Val	Asp	Ser	Arg	Lys	Gln	Ser	Arg	Thr	Glu	Pro				
				245					250					255					
Arg	Thr	Ile	Leu	Val	Pro	Pro	Ala	Asp	Asn	Glu	Ser	Ser	Ala	Ser	Ile				
			260					265					270						
Val	Pro	Arg	Thr	Lys	Lys	Pro	Leu	Pro	Gly	Asp	His	Leu	Val	Ser	Thr				
		275					280					285							
Gln	Gln	Ile	Asp	Gly	Val	Gly	Met	Pro	Ala	Thr	Asn								
	290					295					300								

<210> SEQ ID NO 37  
 <211> LENGTH: 937  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 37

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gttccgctcc gccacaccaa aaagaaacaa aagcctacgg cgatcgatcg agaagctggt      60
catcgtcagg atgtcatcgc cgcagtcagc agcgcgcgcc gtgaagctga tcacggcggt      120
cggcagcccc ttgcccacc gcgtggagggt ggcgctcgct ctcaaggggg tgccgtacga      180
gctggtcgtg gaggacctag ccaacaagag cgagctgctg ctcacgcaca acccagtcca      240
ccagtcggtc cctgtcctcc tccacggtga cgcgctgtc tgcgagtccc tcgtcatcgt      300
cgagtacgtc gacgagacct tccaccatgg cgcggcgccg gggatcctcc cggccgacct      360
ctacgaccgc gccaccgccc gcttctgggc tgacttcatc gacaacaagt gcttgaagcc      420
gatgtggctg tcgatgtgga cggacggcga ggcgcaggcg cggttcgtca gggagacgaa      480
ggagagcctg ggggtgctgg acgcgcaact ccaggggaag aggttcttcg ccggcgacgc      540
gctcggcttc gtcgacctcg ccgcctgcac gctggctcac tggctaggcg tgctggagga      600
agtggccgga gtgcacctga tagcggcgga cggcgagtag cccgctctgc gccgctgggc      660
caaggagtag gtctccgatg aggtcgtgag ccggtcgtg ccggacaggg acgagctcgt      720
cgccttcttc accgccagca aggagaggta caagtctgtg gtcagggcag aggtggagcg      780
acattgatcg ctaaaaatga cgctggttgt tgaactctgt acgttctctt gtagtcgaat      840
aatgtgtgga tcgttatgta tacgtactac gtatttcatc gtgttatata catctaataa      900
gacatgcaag ctcgatcaaa aaaaaaaaaa aaaaaaaa      937
    
```

<210> SEQ ID NO 38  
 <211> LENGTH: 238  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 38

Met	Ser	Ser	Pro	Gln	Ser	Ala	Ala	Pro	Pro	Val	Lys	Leu	Ile	Thr	Ala				
1				5					10					15					
Phe	Gly	Ser	Pro	Phe	Ala	His	Arg	Val	Glu	Val	Ala	Leu	Ala	Leu	Lys				
			20					25						30					
Gly	Val	Pro	Tyr	Glu	Leu	Val	Val	Glu	Asp	Leu	Ala	Asn	Lys	Ser	Glu				
		35					40					45							

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Leu Leu Leu Thr His Asn Pro Val His Gln Ser Val Pro Val Leu Leu  
     50                                  55                                  60  
 His Gly Asp Arg Ala Val Cys Glu Ser Leu Val Ile Val Glu Tyr Val  
   65                                  70                                  75                                  80  
 Asp Glu Thr Phe His His Gly Ala Ala Pro Gly Ile Leu Pro Ala Asp  
                                   85                                  90                                  95  
 Pro Tyr Asp Arg Ala Thr Ala Arg Phe Trp Ala Asp Phe Ile Asp Asn  
                                   100                                  105                                  110  
 Lys Cys Leu Lys Pro Met Trp Leu Ser Met Trp Thr Asp Gly Glu Ala  
                                   115                                  120                                  125  
 Gln Ala Arg Phe Val Arg Glu Thr Lys Glu Ser Leu Gly Val Leu Asp  
   130                                  135                                  140  
 Ala Gln Leu Gln Gly Lys Arg Phe Phe Ala Gly Asp Ala Leu Gly Phe  
   145                                  150                                  155                                  160  
 Val Asp Leu Ala Ala Cys Thr Leu Ala His Trp Leu Gly Val Leu Glu  
                                   165                                  170                                  175  
 Glu Val Ala Gly Val His Leu Ile Ala Ala Asp Gly Glu Tyr Pro Ala  
                                   180                                  185                                  190  
 Leu Arg Arg Trp Ala Lys Glu Tyr Val Ser Asp Glu Val Val Ser Arg  
   195                                  200                                  205  
 Ser Leu Pro Asp Arg Asp Glu Leu Val Ala Phe Phe Thr Ala Ser Lys  
   210                                  215                                  220  
 Glu Arg Tyr Lys Ser Trp Val Arg Ala Glu Val Glu Arg His  
   225                                  230                                  235

<210> SEQ ID NO 39  
 <211> LENGTH: 773  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 39

cgggagccga cgacctgaag gtgctgggcc tgtggacgag cccgttcgtg atccgggtcc 60  
 gcatcgtgct caacctcaag ggcctggcgt acgagtacgt ggaggacgac ctcggcaaca 120  
 agagcgcgct cctgctcagc tccaaccggy tgcacaagac cgtgcccgtg ctgctccacg 180  
 cgggtcgcgc cgtaaacgag tcccagatca tctgcagta catcgacgag gtctgggcgg 240  
 ggaccgggcc ggccgtgctg ccgcgcgacc cctatgagcg cgcggccgcy cggttctggg 300  
 cggcctacat cgacgacaag gtgaagtccg cgtggctggg catgctgttc gactgcaggg 360  
 acgaggggga gcgggcggag gcggtggcgc gggccggcga ggcgctcggg acgctggagg 420  
 gcgcgctcag ggggaagccc ttcttcggcg ggcgagcgt cggcttcgtg gacgcccgtc 480  
 tcggcgggta cctcggctgg ttccggggccg tcggcaggat catcgccgc aggctgatcg 540  
 acccgactaa gacgccgctg ctggccgctg gggaggaccg gttccgccc gccgacgtgg 600  
 ccaagggcgt cgtaccggac gacgtcgaca agatgctcgc gttcctggag accctgctcg 660  
 cgaactacta ctccaagtga ctgtactgag agcgaactac tgctccaagt gactaaataa 720  
 gaagggctgc ttaattaata attacagtat ataaaaaaaa aaaaaaaaaa aaa 773

<210> SEQ ID NO 40  
 <211> LENGTH: 225  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 40



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Gly	Ala	Asp	Asp	Leu	Lys	Val	Leu	Gly	Leu	Trp	Thr	Ser	Pro	Phe	Val
1				5					10					15	
Ile	Arg	Val	Arg	Ile	Val	Leu	Asn	Leu	Lys	Gly	Leu	Ala	Tyr	Glu	Tyr
		20						25					30		
Val	Glu	Asp	Asp	Leu	Gly	Asn	Lys	Ser	Ala	Leu	Leu	Leu	Ser	Ser	Asn
		35					40						45		
Pro	Val	His	Lys	Thr	Val	Pro	Val	Leu	Leu	His	Ala	Gly	Arg	Pro	Val
	50					55					60				
Asn	Glu	Ser	Gln	Ile	Ile	Leu	Gln	Tyr	Ile	Asp	Glu	Val	Trp	Ala	Gly
65				70					75						80
Thr	Gly	Pro	Ala	Val	Leu	Pro	Arg	Asp	Pro	Tyr	Glu	Arg	Ala	Ala	Ala
				85					90					95	
Arg	Phe	Trp	Ala	Ala	Tyr	Ile	Asp	Asp	Lys	Val	Lys	Ser	Ala	Trp	Leu
			100					105					110		
Gly	Met	Leu	Phe	Glu	Cys	Arg	Asp	Glu	Gly	Glu	Arg	Ala	Glu	Ala	Val
		115					120					125			
Ala	Arg	Ala	Gly	Glu	Ala	Leu	Gly	Thr	Leu	Glu	Gly	Ala	Leu	Arg	Gly
	130					135					140				
Lys	Pro	Phe	Phe	Gly	Gly	Asp	Gly	Val	Gly	Phe	Val	Asp	Ala	Val	Leu
145				150						155					160
Gly	Gly	Tyr	Leu	Gly	Trp	Phe	Gly	Ala	Val	Gly	Arg	Ile	Ile	Gly	Arg
			165						170					175	
Arg	Leu	Ile	Asp	Pro	Thr	Lys	Thr	Pro	Leu	Leu	Ala	Ala	Trp	Glu	Asp
			180					185						190	
Arg	Phe	Arg	Ala	Ala	Asp	Val	Ala	Lys	Gly	Val	Val	Pro	Asp	Asp	Val
		195					200					205			
Asp	Lys	Met	Leu	Ala	Phe	Leu	Glu	Thr	Leu	Leu	Ala	Asn	Tyr	Tyr	Ser
	210					215					220				
Lys															
225															

<210> SEQ ID NO 41  
 <211> LENGTH: 860  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 41

agaaaaaagc	ataagctgag	catccatcaa	tggcggatgc	tggcaacgag	gccgagggtc	60
tgacgctggt	ggcctgcac	gtgagccct	tgcggttgcg	cgtgcgcatg	gcgctgagcc	120
tcaagggcct	gagctacgag	tacatcgagc	aggacctggt	ccacaagggc	gagctcctcc	180
tcagctcaaa	ccccgtgcac	aagaaggtgc	cogtgctcat	ccaccacggc	aagcccatct	240
gcgagtccct	cgccgtcgtg	gagtacgtcg	atgaggtctg	gcccggcgcc	gccgccacca	300
tcctccccgc	cgacccccac	ggtcgcgcca	cgctcgtctt	ctgggcccgc	tacatcgacg	360
gcaagctggt	tccggcgtgg	acagggatca	tgaaggcggc	gacggaggaa	gcgagggcgg	420
ataagctgag	ggagacgcac	gccgcggtcc	tcaacctgga	gaaggccttc	gccgagatca	480
gctctagctc	cagcaacgac	ggcgcggcct	tcttcggcgg	cgactccgtc	gggtacctgg	540
acctcgcgct	cgggtgctcc	ctgccgtggt	toggggcgct	gcgcgcatg	ctcggcgtcg	600
agatcatcga	cgccgccag	gctccgctcc	tgggtggcgtg	ggccgagcga	tttggggaga	660
ccccggtggc	caaggaggtg	ctgccgcagc	cggacgagggc	tgtggcctac	gccaagaaga	720
ttcaggccta	ctgggcttct	gctaagaact	gatgagcacc	gaatcctgtc	atgatgaaat	780

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tgaagcagca atacttgtat aacactccaa tcatggtgaa taaaggcctc taaactgttg 840  
 gttaataaaa aaaaaaaaaa 860

<210> SEQ ID NO 42  
 <211> LENGTH: 240  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 42

Met Ala Asp Ala Gly Asn Glu Ala Glu Gly Leu Thr Leu Leu Gly Leu  
 1 5 10 15  
 His Val Ser Pro Phe Ala Leu Arg Val Arg Met Ala Leu Ser Leu Lys  
 20 25 30  
 Gly Leu Ser Tyr Glu Tyr Ile Glu Gln Asp Leu Phe His Lys Gly Glu  
 35 40 45  
 Leu Leu Leu Ser Ser Asn Pro Val His Lys Lys Val Pro Val Leu Ile  
 50 55 60  
 His His Gly Lys Pro Ile Cys Glu Ser Leu Ala Val Val Glu Tyr Val  
 65 70 75 80  
 Asp Glu Val Trp Pro Gly Ala Ala Ala Thr Ile Leu Pro Ala Asp Pro  
 85 90 95  
 His Gly Arg Ala Thr Ala Arg Phe Trp Ala Ala Tyr Ile Asp Gly Lys  
 100 105 110  
 Leu Phe Pro Ala Trp Thr Gly Ile Met Lys Ala Ala Thr Glu Glu Ala  
 115 120 125  
 Arg Ala Asp Lys Leu Arg Glu Thr His Ala Ala Val Leu Asn Leu Glu  
 130 135 140  
 Lys Ala Phe Ala Glu Ile Ser Ser Ser Ser Ser Asn Asp Gly Ala Ala  
 145 150 155 160  
 Phe Phe Gly Gly Asp Ser Val Gly Tyr Leu Asp Leu Ala Leu Gly Cys  
 165 170 175  
 Ser Leu Pro Trp Phe Gly Ala Leu Arg Ala Met Leu Gly Val Glu Ile  
 180 185 190  
 Ile Asp Ala Ala Gln Ala Pro Leu Leu Val Ala Trp Ala Glu Arg Phe  
 195 200 205  
 Gly Glu Thr Pro Val Ala Lys Glu Val Leu Pro Gln Pro Asp Glu Ala  
 210 215 220  
 Val Ala Tyr Ala Lys Lys Ile Gln Ala Tyr Trp Ala Ser Ala Lys Asn  
 225 230 235 240

<210> SEQ ID NO 43  
 <211> LENGTH: 1228  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 43

gtcgtcactg ctgaccatag gtggccggcc ggccgaacaa accctcggca cgatcgctg 60  
 cctccataaa tctccctctt cacttcaggc gaaaaggatc aaccaaacc tctaatccat 120  
 ttcggcattt ccaacgcctt cgccctacca gccacgtcgc ttcgaggccg atcgaccgag 180  
 cagctgggtg caatggcggc ggcggcggag gtcgtgctgc tggacttctg ggtgagcccc 240  
 ttcgggcagc gctgccgat cgcgctggcg gagaagggcg tggcctacga gtaccgcgag 300  
 caggacctcc tggacaaggc cgagctgctc ctccgctcca accccatcca caagaagatc 360  
 cccgtcctgc tccacgcccg caggcccgtc tgcgagtcgc tcgtcatcct ccagtacatc 420



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gacgaggcct ggccggacgt cgcgccgctc ctcccgaagg acgacccta cgcccgcgcg 480
caggcgcggtt tctgggcccga ttacatcgac aagaagatct atgacagcca gactcggctg 540
tggaagtctg agggcgaggc gcgggagcag gcgaagaagg acctggtgga ggtcctggag 600
acctggaggg ggagctcgcc gacaagcctt tcttcggcgg cggcgcctc ggcttcgtgg 660
acgtggctct ggtgcccttc acgtcctggt tcctcgccta cgagaagctg ggcgggttca 720
gcgtccagga gactgcccc aggatcgtgg cctgggcccgc gcgctgcagg gagcgggaga 780
gcgtggccaa ggccatgtcc gaccctgcca aggtgctcga gttcgtccag ttcctccaga 840
gcaagtctcg ggccaagtga tcggaagcat tgcgtgtgct gctagcctgc tatgccctat 900
gcaggccagg ctggtgcttt gatctgctcg atcagctcta tgcccatgct agcgttgcag 960
agcgcagttg atgtgtgatg tgtctggttg gttgtagctg ctctttgcct ggtttcgtac 1020
gtcagtgtaa ggtttcaggt tttcagtgtc tggggtagct ctgctgtgccc cttgccctg 1080
ccccctacct agcggctctt gagctcttcg gctcgcagc aataaagttg cagaggcttt 1140
agctaaaagt ttctgtattt tttagttgac gattattggt ccaatgtatt cggaatttt 1200
gttctctcta aaaaaaaaaa aaaaaaaaaa 1228

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<210> SEQ ID NO 44
<211> LENGTH: 230
<212> TYPE: PRT
<213> ORGANISM: maize

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<400> SEQUENCE: 44

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Met Ala Ala Ala Ala Glu Val Val Leu Leu Asp Phe Trp Val Ser Pro
 1           5           10           15
Phe Gly Gln Arg Cys Arg Ile Ala Leu Ala Glu Lys Gly Val Ala Tyr
          20           25           30
Glu Tyr Arg Glu Gln Asp Leu Leu Asp Lys Gly Glu Leu Leu Leu Arg
          35           40           45
Ser Asn Pro Ile His Lys Lys Ile Pro Val Leu Leu His Ala Gly Arg
          50           55           60
Pro Val Cys Glu Ser Leu Val Ile Leu Gln Tyr Ile Asp Glu Ala Trp
          65           70           75           80
Pro Asp Val Ala Pro Leu Leu Pro Lys Asp Asp Pro Tyr Ala Arg Ala
          85           90           95
Gln Ala Arg Phe Trp Ala Asp Tyr Ile Asp Lys Lys Ile Tyr Asp Ser
          100          105          110
Gln Thr Arg Leu Trp Lys Phe Glu Gly Glu Ala Arg Glu Gln Ala Lys
          115          120          125
Lys Asp Leu Val Glu Val Leu Glu Thr Trp Arg Gly Ser Ser Pro Thr
          130          135          140
Ser Leu Ser Ser Ala Ala Ala Pro Ser Ala Ser Trp Thr Trp Leu Trp
          145          150          155          160
Cys Pro Ser Arg Pro Gly Ser Ser Pro Thr Arg Ser Trp Ala Gly Ser
          165          170          175
Ala Ser Arg Ser Thr Ala Pro Gly Ser Trp Pro Gly Pro Arg Ala Ala
          180          185          190
Gly Ser Gly Arg Ala Trp Pro Arg Pro Cys Pro Thr Leu Pro Arg Cys
          195          200          205
Ser Ser Ser Ser Ser Ser Ser Arg Ala Ser Ser Gly Pro Ser Asp Arg
          210          215          220
Lys His Cys Val Cys Cys

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225

230

<210> SEQ ID NO 45  
 <211> LENGTH: 840  
 <212> TYPE: DNA  
 <213> ORGANISM: maize  
 <400> SEQUENCE: 45

```

caagctaagc aagtgccaac caacgagtag caggaaacat gtctccgccc gtcaagatcc    60
tcggccacta cgcgagcccg tactcgcacc gcgctcgaggc cgctctgcgg ctcaagggcg    120
tgccgtacga gctgggtccag gaagacctgg gcaacaagag cgagctgctg ctcgccaaga    180
accctgtcca caagaagggtg cccgtgctcc tccatggcga cagggccgctc tgcgagtccc    240
tcctcatcgt cgagtacgtc gacgaggcct togacggggc gtccatcctg ccggccgacc    300
cccacgaccg tgccgtcgcc cgtttctggg cgaacttctt ggacaccaag ttctcccagc    360
cgtttctggct ggcgtactgg gcggagggcg aggcgcagaa ggccgtggtg aaggaggcca    420
aggagaacct ggcgctcctg gaggcgcagc tcggcgggaa gaggttcttc ggcggcgaca    480
cgcccgggta cctcgacata gccgcgtgca cgttgggtcc ttggatcggc gtgctcgagg    540
aggtgactgg agtggccttg ctggacgccg acgagttccc cgctctatgc cagtgggcca    600
gggactacag ctccagtga a gcgctcaggc catgcctgcc ggacagggac cgactcgttg    660
cctacttcac cgagaacaag gagaagtaca agacatttgc caaggcaacg ttgcatcagt    720
agctgctagt tgggtgcaaa ccgcttgttt atctctgtgt ggaataatgt atacgtacgt    780
gctccctcga tatcaataa atcagctacc ggagttgact gtagtcaaaa aaaaaaaaaa    840
  
```

<210> SEQ ID NO 46  
 <211> LENGTH: 227  
 <212> TYPE: PRT  
 <213> ORGANISM: maize  
 <400> SEQUENCE: 46

```

Met Ser Pro Pro Val Lys Ile Leu Gly His Tyr Ala Ser Pro Tyr Ser
  1           5           10           15
His Arg Val Glu Ala Ala Leu Arg Leu Lys Gly Val Pro Tyr Glu Leu
           20           25           30
Val Gln Glu Asp Leu Gly Asn Lys Ser Glu Leu Leu Leu Ala Lys Asn
           35           40           45
Pro Val His Lys Lys Val Pro Val Leu Leu His Gly Asp Arg Ala Val
           50           55           60
Cys Glu Ser Leu Leu Ile Val Glu Tyr Val Asp Glu Ala Phe Asp Gly
           65           70           75           80
Pro Ser Ile Leu Pro Ala Asp Pro His Asp Arg Ala Val Ala Arg Phe
           85           90           95
Trp Ala Asn Phe Leu Asp Thr Lys Phe Ser Gln Pro Phe Trp Leu Ala
           100          105          110
Tyr Trp Ala Glu Gly Glu Ala Gln Lys Ala Val Val Lys Glu Ala Lys
           115          120          125
Glu Asn Leu Ala Leu Leu Glu Ala Gln Leu Gly Gly Lys Arg Phe Phe
           130          135          140
Gly Gly Asp Thr Pro Gly Tyr Leu Asp Ile Ala Ala Cys Thr Leu Gly
           145          150          155          160
Pro Trp Ile Gly Val Leu Glu Glu Val Thr Gly Val Ala Leu Leu Asp
           165          170          175
  
```





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Tyr Glu Arg Ala Val Ala Arg Phe Trp Ala Lys Tyr Val Asp Gly Lys  
                   100                                  105                                  110  
 Leu His Gly Met Met Val Lys Ala Leu Met Gly Ala Thr Glu Glu Glu  
                   115                                  120                                  125  
 Arg Ala Thr Ala Thr Val Asp Ala Leu Ala Ala Met Asp Thr Leu Glu  
                   130                                  135                                  140  
 Gly Ala Phe Ala Glu Cys Ser Gly Gly Lys Lys Phe Phe Ala Gly Asp  
                   145                                  150                                  155                                  160  
 Ala Pro Gly Tyr Leu Asp Val Ala Leu Gly Gly Phe Ile Gly Trp Leu  
                                   165                                  170                                  175  
 Arg Ala Trp Asp Lys Val Gly Gly Val Lys Leu Leu Asp Ala Gly Arg  
                   180                                  185                                  190  
 Val Pro Arg Leu Ala Thr Trp Ala Glu Arg Phe Ala Ala Leu Asp Val  
                   195                                  200                                  205  
 Ala Lys Glu Val Ile Pro Asp Pro Asp His Ile Ala Glu Phe Ala Lys  
                   210                                  215                                  220  
 Val Leu Gln Ala Arg Ser Ala Ala Ala Ala Thr Ser Asn  
                   225                                  230                                  235

<210> SEQ ID NO 49  
 <211> LENGTH: 756  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 49

cggcgggtgag gctggtgggc tccttcgcca gcccgttcgt ccaccgcgcc gaggtggccc 60  
 tgcgcctcaa aggggtgccc tacgagctca tcctggagga cctgggcaac aagagcgagc 120  
 tgctgctggc acacaacccc gtgcacaaac tcgtgcccgt gtcctccac ggcgacaggg 180  
 ccatctccga gtcgctcgtc atcctcgagt acgtcgacga ggccttcgac gggccgcctc 240  
 tcctccccgc ggaacccac gcgagggcgg acgcgcggtt ctggggccac ttcacgcacc 300  
 aaaagttcgc gcggccgttc tggatgtcgt tctggacgga cgacgaggag cgcagggagg 360  
 ctatggcgaa ggaggccaag gagaacctgg ctctgctcga ggcgcagctc agggggcaga 420  
 gggttcttcgg cggcgaggcc atcggcttcg tcgacatcgc cgctgtgcg ctggcgcact 480  
 gggtcggggg catcgaggag gctgccgggg tggctcctcgt cggcggcgag gagttcccag 540  
 cgctccgcga gtgggcccgc gcctacgtca acgacgccac cgtgaagcag tgcttgagga 600  
 gccgcgacga gtcgctcgat tactttctccg ccaggaagga gatgtacttg ctgagagcga 660  
 gggccactcc gcgcagctga tctggacccc atgtttcctt ccgttcgcaa taagccaata 720  
 ataaagacta gtttggtaaa aaaaaaaaaa aaaaaa 756

<210> SEQ ID NO 50  
 <211> LENGTH: 225  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 50

Ala Val Arg Leu Val Gly Ser Phe Ala Ser Pro Phe Val His Arg Ala  
   1                  5                                  10                                  15  
 Glu Val Ala Leu Arg Leu Lys Gly Val Pro Tyr Glu Leu Ile Leu Glu  
                   20                                  25                                  30  
 Asp Leu Gly Asn Lys Ser Glu Leu Leu Leu Ala His Asn Pro Val His  
                   35                                  40                                  45



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Lys Leu Val Pro Val Leu Leu His Gly Asp Arg Ala Ile Ser Glu Ser  
     50                                    55                                    60  
 Leu Val Ile Leu Glu Tyr Val Asp Glu Ala Phe Asp Gly Pro Pro Leu  
     65                                    70                                    75                                    80  
 Leu Pro Ala Glu Pro His Ala Arg Ala Asp Ala Arg Phe Trp Ala His  
                                     85                                    90                                    95  
 Phe Ile Asp Gln Lys Phe Ala Arg Pro Phe Trp Met Ser Phe Trp Thr  
                                     100                                    105                                    110  
 Asp Asp Glu Glu Arg Arg Glu Ala Met Ala Lys Glu Ala Lys Glu Asn  
                                     115                                    120                                    125  
 Leu Ala Leu Leu Glu Ala Gln Leu Arg Gly Gln Arg Phe Phe Gly Gly  
     130                                    135                                    140  
 Glu Ala Ile Gly Phe Val Asp Ile Ala Ala Cys Ala Leu Ala His Trp  
     145                                    150                                    155                                    160  
 Val Gly Val Ile Glu Glu Ala Ala Gly Val Val Leu Val Gly Gly Glu  
                                     165                                    170                                    175  
 Glu Phe Pro Ala Leu Arg Glu Trp Ala Asp Ala Tyr Val Asn Asp Ala  
                                     180                                    185                                    190  
 Thr Val Lys Gln Cys Leu Arg Ser Arg Asp Glu Leu Val Asp Tyr Phe  
                                     195                                    200                                    205  
 Ser Ala Arg Lys Glu Met Tyr Leu Leu Arg Ala Arg Ala Thr Pro Arg  
     210                                    215                                    220  
 Ser  
 225

<210> SEQ ID NO 51  
 <211> LENGTH: 967  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 51

```

cagccacggc gagcaagcga tgatgagcgg cggcgcgggtg aaggtgatcg gcgccctgga      60
cagcccgttc agccaccgcg cggaggcggc gctgcgcctc aagggagtcc cctacgagct      120
tgtcctggag aaggacctgc gcgacaaaag cgagctgctg ctgcggcaca accccgtcca      180
caagaaggtg cccgtgctcc ttcacggcgg ccgcccgcgc gtctgcgagt cgctcgtcat      240
cgtcgagtac gttgacgagg cattccgcgg cccgccactc ctccccgccg acccctccgc      300
ccgcgcgcgc gcccgcttct gggcccgtt catcgacgac aagtgctcga cgcccttctg      360
gctggcgatg tggacggagg gcgaggcgca gagggggttc gtgaaggaga tcaaggagaa      420
cctgaagctg ctggaggggc aggtgaaggc caagcggttc ttcggcggcg gcgacgtggg      480
ctacctgac gtcgccgcca gcgtgttcgc gcaactggctt ccggtctgcg aggaggtcgc      540
gggcgtcagc ctggtcacgg ccgaggagta cccggacctg tgccgggtggg cgagggagta      600
cacctcccac gacgccgtca agcggtgctt gcctggcagg gaggagctgc tcgcccgttt      660
cagcgcagg aaggactcgt ttgtggccgc ggcgagggtca atggcgcgg cgccggagaa      720
gtaatctatg ggaattcaac tgggtgcatg gatagcataa atttaagtat tgtgacaagt      780
ggtcaggact gctgtcacgt accgtcgagc ccgggagata tgtactctcc ggctcaggca      840
aatgccatct gtctgatgc atgtgttttg ttctactttt gtttgactgc ttgttgaata      900
aaaaaagata tccggttggt ttcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      960
aaaaaaa                                         967
  
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<210> SEQ ID NO 52  
 <211> LENGTH: 234  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 52

Met Met Ser Gly Gly Ala Val Lys Val Ile Gly Ala Leu Asp Ser Pro  
 1 5 10 15  
 Phe Ser His Arg Ala Glu Ala Ala Leu Arg Leu Lys Gly Val Pro Tyr  
 20 25 30  
 Glu Leu Val Leu Glu Lys Asp Leu Arg Asp Lys Ser Glu Leu Leu Leu  
 35 40 45  
 Arg His Asn Pro Val His Lys Lys Val Pro Val Leu Leu His Gly Gly  
 50 55 60  
 Arg Arg Ala Val Cys Glu Ser Leu Val Ile Val Glu Tyr Val Asp Glu  
 65 70 75 80  
 Ala Phe Arg Gly Pro Pro Leu Leu Pro Ala Asp Pro Ser Ala Arg Ala  
 85 90 95  
 Ala Ala Arg Phe Trp Ala Arg Phe Ile Asp Asp Lys Cys Ser Thr Pro  
 100 105 110  
 Phe Trp Leu Ala Met Trp Thr Glu Gly Glu Ala Gln Arg Gly Phe Val  
 115 120 125  
 Lys Glu Ile Lys Glu Asn Leu Lys Leu Leu Glu Gly Gln Val Lys Gly  
 130 135 140  
 Lys Arg Phe Phe Gly Gly Gly Asp Val Gly Tyr Leu Asp Val Ala Ala  
 145 150 155 160  
 Ser Val Phe Ala His Trp Leu Pro Val Cys Glu Glu Val Ala Gly Val  
 165 170 175  
 Ser Leu Val Thr Ala Glu Glu Tyr Pro Asp Leu Cys Arg Trp Ala Arg  
 180 185 190  
 Glu Tyr Thr Ser His Asp Ala Val Lys Arg Cys Leu Pro Gly Arg Glu  
 195 200 205  
 Glu Leu Leu Ala Arg Phe Ser Ala Arg Lys Asp Ser Phe Val Ala Ala  
 210 215 220  
 Ala Arg Ser Met Ala Pro Ala Pro Glu Lys  
 225 230

<210> SEQ ID NO 53  
 <211> LENGTH: 1100  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 53

acctcagaca ctctgcatat atcctctgcg cgtgtatctc gtcgaacaga gccgaaagct 60  
 ggagcttcca atggcgggag gcaacgacct gaaggtgctc ggcgtgtgga cgagcccgtt 120  
 cgtgatccgg gtccgcatcg tgctcaacct gaagggcctg gcgtacgagt acgtggagga 180  
 ggacctcggc aacaagagcg cgctcctcct gggatccaac ccggtgcaca agagcgtgcc 240  
 ggtgctcctc cacgccggcc gcgccataaa cgagtcccag gtcatacctgc agtacatcga 300  
 cgaggtgtgg gcggggacgg ggccggccgt gcttccggcc gaccctacg agcgcgcggt 360  
 ggcgcggttc tggggcgcgt acatcgacga caaggtggag tcggcgtggc tggggatgct 420  
 gttcaggtgc gcgaacgagg aggagagggc ggcggcggtg gcgcgcgccc gcgaggcgct 480  
 cgacgcgctg gagggcgcgt tccgggactg ctccaggggg aggccgttct tcggcggcga 540  
 cgacatcggg ttcgtggacg ccgttctcgg cgggtacctc ggctggttcg gggccgtcgg 600



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caggatcatc gggagcaggc tcatcgaccc ggcccggacg ccgctgctgg ccgctgggga 660
ggaccggttc cgcgccgccc acgtggccaa gggcgtcgtg cccgacgacc tcgacaagat 720
gctcgcgttc ctgcagaccc tgcgcgctat gaactacgcc aagtgagagt gtttcgtcgc 780
atgaacgtgt gccgtgccgt gcacgaccta tgatcagttc atgtcgatac gtctatcact 840
cagttttgct tcttccgtca ataatcggtg tgctgaatac atgtacaaca gctgcctata 900
atthtgcttc tttcttctaa tacctccggt ttttaatttga tagttcaact ttataataac 960
aatgtcaaaa tttaaaaaaaa aaatcggaag gagtattttt ttaatacatc caagtccatc 1020
caataaaagt gctcgtgggg ctttctatta aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1080
aaaaaaaaaa aaaaaaaaaa 1100

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<210> SEQ ID NO 54
<211> LENGTH: 231
<212> TYPE: PRT
<213> ORGANISM: maize

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<400> SEQUENCE: 54

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Met Ala Gly Gly Asn Asp Leu Lys Val Leu Gly Val Trp Thr Ser Pro
 1          5          10          15
Phe Val Ile Arg Val Arg Ile Val Leu Asn Leu Lys Gly Leu Ala Tyr
          20          25          30
Glu Tyr Val Glu Glu Asp Leu Gly Asn Lys Ser Ala Leu Leu Leu Gly
          35          40          45
Ser Asn Pro Val His Lys Ser Val Pro Val Leu Leu His Ala Gly Arg
          50          55          60
Ala Ile Asn Glu Ser Gln Val Ile Leu Gln Tyr Ile Asp Glu Val Trp
          65          70          75          80
Ala Gly Thr Gly Pro Ala Val Leu Pro Ala Asp Pro Tyr Glu Arg Ala
          85          90          95
Val Ala Arg Phe Trp Gly Ala Tyr Ile Asp Asp Lys Val Glu Ser Ala
          100          105          110
Trp Leu Gly Met Leu Phe Arg Cys Ala Asn Glu Glu Glu Arg Ala Ala
          115          120          125
Ala Val Ala Arg Ala Arg Glu Ala Leu Asp Ala Leu Glu Gly Ala Phe
          130          135          140
Arg Asp Cys Ser Arg Gly Arg Pro Phe Phe Gly Gly Asp Asp Ile Gly
          145          150          155          160
Phe Val Asp Ala Val Leu Gly Gly Tyr Leu Gly Trp Phe Gly Ala Val
          165          170          175
Gly Arg Ile Ile Gly Ser Arg Leu Ile Asp Pro Ala Arg Thr Pro Leu
          180          185          190
Leu Ala Ala Trp Glu Asp Arg Phe Arg Ala Ala Asp Val Ala Lys Gly
          195          200          205
Val Val Pro Asp Asp Leu Asp Lys Met Leu Ala Phe Leu Gln Thr Leu
          210          215          220
Arg Ala Met Asn Tyr Ala Lys
          225          230

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<210> SEQ ID NO 55
<211> LENGTH: 934
<212> TYPE: DNA
<213> ORGANISM: maize

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&lt;400&gt; SEQUENCE: 55

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acgacggaaa cagtagtgct gccagtagag agctctcaga actcgggaaa aaaatgtcag    60
aggccgccgt gcgtgtgatc ggcctatggc cgagcccgtt cgtgatccgc gtcctgatcg    120
ccctgaagct gaagggcgtc gagttcgagt tcgtggagga ggtggggtggc aggaagagcg    180
agctgctgct gaggtcgaac ccggtgcaca agaagatccc cgtcctgctc caccacggca    240
agcccatctc cgagtctctg atcgctcgtcc agtacatcga cgaggtctgg tcctccggcg    300
cgccggcctt cctccccgtc gacgctcacg cccgcgccgt ccagcggttc tgggcgcagt    360
acgtcgacga caagctgcct tgggcgatcc gcatactgaa gggaacggac gacgggggca    420
tggagcaggc ggcggggcag ctgtccgcgg ccctgcagct cctagaggag gctttcgcgc    480
agctcagcca ggggaagcgc tacttcggcg gggacagcgt cgggtacctg gacatcgctc    540
tgggtgctgca tgtcggctgg gtgaaggcgg tggagaagat cgccgggggtc accctgctgg    600
acaaggccaa ggtcccgaac ctggtggcgt gggctgatcg tctgtgtgcc caccggccg    660
tggtcgacgc catccctgac gcggacaagt tcggtgagtt cagcgtcacc tatggctcct    720
tttcgaagcc tatcaatgct cccgccaagt gagcaaaaag ggtccgtgca tgctttcgtc    780
atcttcactt tcactgcgcg tgtgccggtg cgtgtcaaaa ttgcatagca aggggattct    840
tctcccacat agatcttctt gtgatcatag tcaccaaata agctctgaaa atgaagattt    900
tctgcccttc caaaggaaaa aaaaaaaaaa aaaa    934

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&lt;210&gt; SEQ ID NO 56

&lt;211&gt; LENGTH: 232

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: maize

&lt;400&gt; SEQUENCE: 56

```

Met Ser Glu Ala Ala Val Arg Val Ile Gly Leu Trp Pro Ser Pro Phe
  1           5           10           15
Val Ile Arg Val Leu Ile Ala Leu Lys Leu Lys Gly Val Glu Phe Glu
          20           25           30
Phe Val Glu Glu Val Val Gly Arg Lys Ser Glu Leu Leu Leu Arg Ser
          35           40           45
Asn Pro Val His Lys Lys Ile Pro Val Leu Leu His His Gly Lys Pro
          50           55           60
Ile Ser Glu Ser Leu Ile Val Val Gln Tyr Ile Asp Glu Val Trp Ser
          65           70           75           80
Ser Gly Ala Pro Ala Phe Leu Pro Val Asp Ala His Ala Arg Ala Val
          85           90           95
Gln Arg Phe Trp Ala Gln Tyr Val Asp Asp Lys Leu Pro Trp Ala Ile
          100          105          110
Arg Ile Leu Lys Gly Thr Asp Asp Gly Gly Met Glu Gln Ala Ala Gly
          115          120          125
Gln Leu Ser Ala Ala Leu Gln Leu Leu Glu Glu Ala Phe Ala Gln Leu
          130          135          140
Ser Gln Gly Lys Arg Tyr Phe Gly Gly Asp Ser Val Gly Tyr Leu Asp
          145          150          155          160
Ile Ala Leu Val Ser His Val Gly Trp Val Lys Ala Val Glu Lys Ile
          165          170          175
Ala Gly Val Thr Leu Leu Asp Lys Ala Lys Val Pro Asn Leu Val Ala
          180          185          190
Trp Ala Asp Arg Leu Cys Ala His Pro Ala Val Val Asp Ala Ile Pro

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195	200	205	
Asp Ala Asp Lys Phe Val Glu Phe Ser Val Thr Tyr Gly Ser Phe Ser			
210	215	220	
Lys Pro Ile Asn Ala Pro Ala Lys			
225	230		

<210> SEQ ID NO 57  
 <211> LENGTH: 960  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 57

aaccgcagct gaagctgctg gccatgtggg cgagcccgtt tgccctacgg gcgaagctag	60
cgctcaactt caagggcctg gcctacgagt acgtagagga ggacctcgc agcaagagcg	120
acctcctgct gagctcgaac ccggtgcaca agaaggtgcc cgtcctcatc cacaacggcg	180
tgcccgtctg tgagtcgcyg gtcacgtgg agtacctcga cgaagtctac agcgccacgg	240
gccccgctt cctccctgcc gaccatacag agcgtgccat ggcgcgcttc tgggcctcat	300
tcatcgacga aaagttcttg gcgtcgtggc taaaggcagg aaggggcaag acggacgagg	360
agaaggccga agggttgaag ctgacactcg cggccgtaga aaccttgaa ggggcgttca	420
tggagtgtc caaggggaag cccttctttg gaggcgatag tgtcggctac ctggacatcg	480
cgctcggggc cctggtagcg tggatgcgcy ccaccgaggc gcgtcatggt ctgaggctct	540
tcgacgcctc cagagtccgc tgctggagaa gtgggtggag cgcttcagcg agctggacga	600
ggtcgtggcg gtcatgccgg acatcgaccg gctagtagag ctcggcaagg tgagggaggc	660
tgctgcygct gcagcagctg ccgtaaacag ctgaacggaa cgcattctgc ggtattgagg	720
cggtaataa gtcggagaaa gatcttgata cctgtgtgta acaagcgaat ggtgtaataa	780
agaatacaat tagggtgtcg tttagttcac atgtcggtaa cgtaatgat aaccgataac	840
attaaatcat gtttgttata agtccaatcg taatcgatct catactacaa aaaaaaaaaa	900
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	960

<210> SEQ ID NO 58  
 <211> LENGTH: 203  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 58

Met Trp Ala Ser Pro Phe Ala Leu Arg Ala Lys Leu Ala Leu Asn Phe	
1	15
Lys Gly Leu Ala Tyr Glu Tyr Val Glu Glu Asp Leu Arg Ser Lys Ser	
20	30
Asp Leu Leu Leu Ser Ser Asn Pro Val His Lys Lys Val Pro Val Leu	
35	45
Ile His Asn Gly Val Pro Val Cys Glu Ser Arg Val Ile Val Glu Tyr	
50	60
Leu Asp Glu Val Tyr Ser Ala Thr Gly Pro Arg Phe Leu Pro Ala Asp	
65	80
Pro Tyr Glu Arg Ala Met Ala Arg Phe Trp Ala Ser Phe Ile Asp Glu	
85	95
Lys Phe Leu Ala Ser Trp Leu Lys Ala Gly Arg Gly Lys Thr Asp Glu	
100	110
Glu Lys Ala Glu Gly Leu Lys Leu Thr Leu Ala Ala Val Glu Thr Leu	
115	125

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Glu Gly Ala Phe Met Glu Cys Ser Lys Gly Lys Pro Phe Phe Gly Gly  
 130 135 140  
 Asp Ser Val Gly Tyr Leu Asp Ile Ala Leu Gly Ala Leu Val Ala Trp  
 145 150 155 160  
 Met Arg Ala Thr Glu Ala Arg His Gly Leu Arg Leu Phe Asp Ala Ser  
 165 170 175  
 Arg Val Arg Cys Trp Arg Ser Gly Trp Ser Ala Ser Ala Ser Trp Thr  
 180 185 190  
 Arg Ser Trp Arg Ser Cys Arg Thr Ser Thr Gly  
 195 200

<210> SEQ ID NO 59  
 <211> LENGTH: 967  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 59

gggctagcta gtcttgacaga ctccgagata cgactagctt gttataacaa gccaaagcaga 60  
 gcgggtgggga aaacaatggc gggcaatgag ggtcttaagg tccttggcct gcaggtgagc 120  
 ccgttcgtgc tccgcgtgtg catggcgttg aacacaaaag gagtgagcta cgagtacgtt 180  
 gaggaggacc tatccaacaa gagtgagctc ctgcttaagt ccaaccggg gcacaagaag 240  
 gtaccctgac tcatccacaa cggttaagccc atctgtgagt cactcgtcat catgcagtac 300  
 gtcgacgagc tgttcgccgg ccggtcgatc ctaccaaccg acccctacga gcgcgccact 360  
 gctcgtcttct gggctgccta cgccgacgac aagttggttc cagcgtggta cggcatggtg 420  
 aaggcccagt cggcggagga gagagcggag aaggtggagg agacgctttc cgcgatccag 480  
 cacatggaag tggccttcgc caagtgtcc ggccggcaacg ccgccttctt cggcggcgac 540  
 tccattggct acgtcgacat cgtgctcggc tccttcttgt tctggttcga ggcggtgagc 600  
 agggtttacg acttgagat cattaacgct agcaatactc cgctcttggc tgcgtgggagc 660  
 gagcggtttg tagggactgt agaagcaaag gaggtggtgc cggtgcccga cgtggacatg 720  
 gccgtacagt gcatcaataa gcttcatgcc cctgcccggc ccataagttc acaatgagtc 780  
 gtgtaagtgt aataaccagg aaaaggtaaa tgggtgaggc ctatggtcca aattccaacc 840  
 gaataatggt caaagcttac attgataggc ttgggttggc gtcacaaat aatgtggttc 900  
 agtcgtctcc tctgcaataa atataaatat gattgtttta gtgtgtaaaa aaaaaaaaaa 960  
 aaaaaaa 967

<210> SEQ ID NO 60  
 <211> LENGTH: 233  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 60

Met Ala Gly Asn Glu Gly Leu Lys Val Leu Gly Leu Gln Val Ser Pro  
 1 5 10 15  
 Phe Val Leu Arg Val Cys Met Ala Leu Asn Thr Lys Gly Val Ser Tyr  
 20 25 30  
 Glu Tyr Val Glu Glu Asp Leu Ser Asn Lys Ser Glu Leu Leu Leu Lys  
 35 40 45  
 Ser Asn Pro Val His Lys Lys Val Pro Val Leu Ile His Asn Gly Lys  
 50 55 60  
 Pro Ile Cys Glu Ser Leu Val Ile Met Gln Tyr Val Asp Glu Leu Phe



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65	70	75	80
Ala Gly Arg Ser	Ile Leu Pro Thr Asp	Pro Tyr Glu Arg Ala Thr	Ala
	85	90	95
Arg Phe Trp Ala Ala Tyr Ala Asp Asp Lys Leu Leu Pro Ala Trp Tyr			
	100	105	110
Gly Met Val Lys Ala Gln Ser Ala Glu Glu Arg Ala Glu Lys Val Glu			
	115	120	125
Glu Thr Leu Ser Ala Ile Gln His Met Glu Val Ala Phe Ala Lys Cys			
	130	135	140
Ser Gly Gly Asn Ala Ala Phe Phe Gly Gly Asp Ser Ile Gly Tyr Val			
	145	150	155
Asp Ile Val Leu Gly Ser Phe Leu Phe Trp Phe Glu Ala Val Arg Arg			
	165	170	175
Val Tyr Asp Leu Glu Ile Ile Asn Ala Ser Asn Thr Pro Leu Leu Ala			
	180	185	190
Ala Trp Ala Glu Arg Phe Val Gly Thr Val Glu Ala Lys Glu Val Val			
	195	200	205
Pro Val Pro Asp Val Asp Met Ala Val Gln Cys Ile Asn Lys Leu His			
	210	215	220
Ala Pro Ala Ala Ala Ile Ser Ser Gln			
	225	230	

<210> SEQ ID NO 61  
 <211> LENGTH: 900  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 61

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ggccaagaac tcgatccgag caaaaaaatg tcggaggccg ccgtgcgagt gatcggccta    60
tggccgagcc cgttcgtgat ccgcgctctg atcgcgctga agctgaagca tgtggagtac    120
gagttcgtgg aggagtggtt gggcagcaag agcgagctgc tgctcgcgtc gaaccggtg    180
cacaagaaga tccccgtcct gctccaccac ggcaagcccc tctccgagtc cctaatcatc    240
gttcagtaca tcgacgaggt ctggtcctcc ggcgcgccgg cggccatcct ccccgccgac    300
ccttacgcgc gcgctgtcca gcggttctgg gcgcagtagc tcgacgaaa gatgcacccg    360
gcgatccgcg tactgaaggg aacgtacgac ggggacaagg agcaggcggc ggggcagctg    420
tccgcggccc tgcagctcct ggaggaggct ttgcgcgagc tcggccaggg gaagcgctac    480
ttcggcgggg acagcgtcgg gtacctggac atcgccctgg tgtcgcacgt cggctgggtg    540
aaggcgggtg agaagatcgc gggggtcact ctgctggagc aggcgaaggt tcccaacctg    600
gtggcgtggg ctgaccggtt gtgcgcccac ccggccgtgg tggacgcgat ccctgacgcc    660
gacaagtctc ttgagttcag cgtgacctat gggtcggttct cttaatccta tcaatgctcc    720
caaagtgagc aaaatggctc cgcattgcgc tttgtgattt tcaactgcga tgtgccggtg    780
catgtcaccg tcaatagcat gtagtttgta tacatgtcct tctgtgaaaa taaagctttg    840
ctgcccgtca aagggaaatc gacataaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    900

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<210> SEQ ID NO 62  
 <211> LENGTH: 225  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 62

Met Ser Glu Ala Ala Val Arg Val Ile Gly Leu Trp Pro Ser Pro Phe

-continued

1	5	10	15
Val Ile Arg	Val Leu Ile	Ala Leu Lys	Leu Lys His Val Glu Tyr Glu
	20	25	30
Phe Val Glu	Glu Val Val	Gly Ser Lys	Ser Glu Leu Leu Leu Ala Ser
	35	40	45
Asn Pro Val	His Lys Lys	Ile Pro Val	Leu Leu His His Gly Lys Pro
	50	55	60
Leu Ser Glu	Ser Leu Ile	Ile Val Gln	Tyr Ile Asp Glu Val Trp Ser
	65	70	75
Ser Gly Ala	Pro Ala Ala	Ile Leu Pro	Ala Asp Pro Tyr Ala Arg Ala
	85	90	95
Val Gln Arg	Phe Trp Ala	Gln Tyr Val	Asp Asp Lys Met His Pro Ala
	100	105	110
Ile Arg Val	Leu Lys Gly	Thr Tyr Asp	Gly Asp Lys Glu Gln Ala Ala
	115	120	125
Gly Gln Leu	Ser Ala Ala	Leu Gln Leu	Leu Glu Glu Ala Phe Ala Gln
	130	135	140
Leu Gly Gln	Gly Lys Arg	Tyr Phe Gly	Gly Asp Ser Val Gly Tyr Leu
	145	150	155
Asp Ile Ala	Leu Val Ser	His Val Gly	Trp Val Lys Ala Val Glu Lys
	165	170	175
Ile Ala Gly	Val Thr Leu	Leu Asp Glu	Ala Lys Val Pro Asn Leu Val
	180	185	190
Ala Trp Ala	Asp Arg Leu	Cys Ala His	Pro Ala Val Val Asp Ala Ile
	195	200	205
Pro Asp Ala	Asp Lys Phe	Val Glu Phe	Ser Val Thr Tyr Gly Ser Phe
	210	215	220
Ser			
225			

<210> SEQ ID NO 63  
 <211> LENGTH: 872  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 63

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ggaaacaaag aaataaagag agacgatggg cggagaagaa ggcggcgacg ggctgaagct    60
gatcgggcag tacgggagcg cgttcgtgac gaggggtgaag cttgctctca gcctcaaggg    120
gctgagctac gagtacgtcg aggaggatct cagaaacaag agcgcgctcc tcctcagctc    180
caaccgggtg cacaaggcgg ttccagtgct gatccacaga ggcaagccta tctgagagtc    240
gcaggtcatc gtgcagtaca tcgacgaggc ctttgccggc atcggcccgc ccctcctccc    300
ggccgacccc tacgaacgct cggtgggccc tttctgggct gccttcattg aagacaagct    360
tgtgtccccg tgggaccgag tgttccgggc gaagacggag gacgagaggg aagaggcgat    420
gaagcagatg cttgcggcag tggacgctct ggagggagca ctgaaggagg ggagaccag    480
acccttcttc ggcggcgaca gcgtcgggta cgtggacgtc gttctgggcg gtgccgtctc    540
gtacgccaag gggcacgacg cgctcttcgg ttccgagctc atcgacgccg ccaagacgcc    600
gctcctggcc gcgtggatgg agcgcttctg cgagctcgac gcggccaagg cggtcctgca    660
ggacgtcgat agagtgttcc agtacggcaa gatgctgatc gccagaatg ctgctgccac    720
tcgtcaggcg tagtgTTTTT ctgatcgatc agcttgtatg tatatgaatt gaacttgtaa    780
aaccaaatcg ttcaagtttg atggtaagtt ccatgttaga aaaaaaaaaa aaaaaaaaaa    840

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aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa

872

<210> SEQ ID NO 64  
 <211> LENGTH: 235  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

&lt;400&gt; SEQUENCE: 64

Met Gly Gly Glu Glu Gly Gly Asp Gly Leu Lys Leu Ile Gly Gln Tyr  
 1 5 10 15  
 Gly Ser Ala Phe Val Thr Arg Val Lys Leu Ala Leu Ser Leu Lys Gly  
 20 25 30  
 Leu Ser Tyr Glu Tyr Val Glu Glu Asp Leu Arg Asn Lys Ser Ala Leu  
 35 40 45  
 Leu Leu Ser Ser Asn Pro Val His Lys Ala Val Pro Val Leu Ile His  
 50 55 60  
 Arg Gly Lys Pro Ile Cys Glu Ser Gln Val Ile Val Gln Tyr Ile Asp  
 65 70 75 80  
 Glu Ala Phe Ala Gly Ile Gly Pro Pro Leu Leu Pro Ala Asp Pro Tyr  
 85 90 95  
 Glu Arg Ser Val Ala Arg Phe Trp Ala Ala Phe Ile Glu Asp Lys Leu  
 100 105 110  
 Val Ser Pro Trp Asp Arg Val Phe Arg Ala Lys Thr Glu Asp Glu Arg  
 115 120 125  
 Glu Glu Ala Met Lys Gln Met Leu Ala Ala Val Asp Ala Leu Glu Gly  
 130 135 140  
 Ala Leu Lys Glu Gly Arg Pro Arg Pro Phe Phe Gly Gly Asp Ser Val  
 145 150 155 160  
 Gly Tyr Val Asp Val Val Leu Gly Gly Ala Val Ser Tyr Ala Lys Gly  
 165 170 175  
 His Asp Ala Leu Phe Gly Ser Glu Leu Ile Asp Ala Ala Lys Thr Pro  
 180 185 190  
 Leu Leu Ala Ala Trp Met Glu Arg Phe Cys Glu Leu Asp Ala Ala Lys  
 195 200 205  
 Ala Val Leu Gln Asp Val Asp Arg Val Val Gln Tyr Gly Lys Met Leu  
 210 215 220  
 Ile Ala Lys Asn Ala Ala Ala Thr Arg Gln Ala  
 225 230 235

<210> SEQ ID NO 65  
 <211> LENGTH: 971  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

&lt;400&gt; SEQUENCE: 65

gtgactgtga tctatactat aagggtgaaca agatctcttt gtctactgta gttgcagcac 60  
 cagcagcagc agcagaagag cagcgcctga gctccagcaa taatggccga gaagggcgtg 120  
 aagggtgttg ggatgtgggc gagcccatg gtgatcaggg tggagtgggc gctgcggctg 180  
 aagggcgtcg agtacgagta cgtcgacgag gacctcgcca acaagagcgc cgacctgctc 240  
 cgccacaacc cggtgaccaa gaaggtgccc gtgctcgtcc acgacggcaa gccggtcgcg 300  
 gagtccacca tcatcgtgga gtacatcgac gaggtctgga agggcggcta ccccatcatg 360  
 ccgggcgacc cctacgagcg cgcccaggcg aggttctggg ccaggttcgc ggaagacaag 420  
 tgcaacgctg ctctgtaccc gatcttcacc ggcaccggcg aggcgcagcg caaggcggtg 480

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cacgaggccc agcagtgcct caagaccctg gagacggcct tggaggggaa gaagttcttc 540
ggcggcgacg ccgtgggcta cctcgacatc gtcgtcgggt ggttcgcgca ctggctcccc 600
gtcatcgagg aggtgaccgg cgccagcgtc gtcacccacg aggagctgcc gctgatgaag 660
gcctggttcg gtcggttcct cgcccttgac gtggtgaagg cggccctgcc cgacagggac 720
aggctcctcg ccgccaacaa ggcccgcctg gagcagctcc tctccgcgta gatatggcta 780
gtaattctgg agcagctagt ttcaccgccc acgctcatat attgctgaat aaggactggt 840
tgcacttttg cacgttggtc agtgcagccc gaggtttgga tgacctctgc ccctctgttc 900
catttcagaa tggtagtccc ataataatgc atatacatca tgcaaaaaaa aaaaaaaaaa 960
aaaaaaaaaa a 971

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<210> SEQ ID NO 66
<211> LENGTH: 222
<212> TYPE: PRT
<213> ORGANISM: maize

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<400> SEQUENCE: 66

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Met Ala Glu Lys Gly Val Lys Val Leu Gly Met Trp Ala Ser Pro Met
 1          5          10          15
Val Ile Arg Val Glu Trp Ala Leu Arg Leu Lys Gly Val Glu Tyr Glu
 20          25          30
Tyr Val Asp Glu Asp Leu Ala Asn Lys Ser Ala Asp Leu Leu Arg His
 35          40          45
Asn Pro Val Thr Lys Lys Val Pro Val Leu Val His Asp Gly Lys Pro
 50          55          60
Val Ala Glu Ser Thr Ile Ile Val Glu Tyr Ile Asp Glu Val Trp Lys
 65          70          75          80
Gly Gly Tyr Pro Ile Met Pro Gly Asp Pro Tyr Glu Arg Ala Gln Ala
 85          90          95
Arg Phe Trp Ala Arg Phe Ala Glu Asp Lys Cys Asn Ala Ala Leu Tyr
100          105          110
Pro Ile Phe Thr Ala Thr Gly Glu Ala Gln Arg Lys Ala Val His Glu
115          120          125
Ala Gln Gln Cys Leu Lys Thr Leu Glu Thr Ala Leu Glu Gly Lys Lys
130          135          140
Phe Phe Gly Gly Asp Ala Val Gly Tyr Leu Asp Ile Val Val Gly Trp
145          150          155          160
Phe Ala His Trp Leu Pro Val Ile Glu Glu Val Thr Gly Ala Ser Val
165          170          175
Val Thr His Glu Glu Leu Pro Leu Met Lys Ala Trp Phe Gly Arg Phe
180          185          190
Leu Ala Leu Asp Val Val Lys Ala Ala Leu Pro Asp Arg Asp Arg Leu
195          200          205
Leu Ala Ala Asn Lys Ala Arg Arg Glu Gln Leu Leu Ser Ala
210          215          220

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<210> SEQ ID NO 67
<211> LENGTH: 1074
<212> TYPE: DNA
<213> ORGANISM: maize

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<400> SEQUENCE: 67

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gctctaacac agcgcaagcc atggcaggac gagtagcgga caaagacca gagctgaagg 60

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tgctcggagt gtggtcgagc ccgttcgta tcagggcccg cgtcgcgcta aacctcaagg 120
gcctggccta ccgatacgtg gaggacaacc tggacagcaa gagcgagctc ctctcgcct 180
ccaaccccgt gcacgggaag gtgccggtgc tcctccacga cggcaggccc gtctgagagt 240
cccgggtcat cgtggagtat atcgacgagg ccttcccggc cagcggcccc tgcctcctcc 300
ccgccgacct gtaccgccgc gccgtcgacc gcttctgggc ctctacgcc gacgacaagc 360
tctttcccac ctggataccc gtctacaacg gcaggacgag cgaggacagg gtcgcggcgg 420
cgaggcaggt cgtggccgtg ctggagaagt ttgagcaggc gttcgatgag tgctccgggt 480
ccgggggcaa ggcgttcttc ggcggggacg ctgctggcct cgtggacgtc gtgctaggcg 540
gcttcctcgg gtggctgagc gcgtctgagg cgatgtgtgg cgtgagggtc atcgaccccg 600
ccaagacgcc gctgctggcg gcgtgggagg accggttcgc cgcgctcgac ggcgtcaggg 660
aggtgatacc tgacgtgcag aggtgctgg agtataacaa gattaggcga gctcgtcgtg 720
ggctgccgta ggtgctgggc cttgggccat ctatctgtca ccatgtggtc agtcaactct 780
aagcaggaga cttgacaag gttgaaagt agttcatgaa ggtgggcaat ctaattagga 840
tgctcatgct ttagctagga gtgccattag ttttctgtt gaaaggcatg tttggttct 900
ctttcctact gaaagagttt gtaatataat ctatgatgct ttgagtttga gaaaagaact 960
atgaataaaa catggatatt ttccgatatt tcagttcaaa ttaaaaaaaaa aaaaaaaaaa 1020
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 1074

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<210> SEQ ID NO 68
<211> LENGTH: 236
<212> TYPE: PRT
<213> ORGANISM: maize

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<400> SEQUENCE: 68

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Met Ala Gly Arg Val Ala Asp Lys Asp Pro Glu Leu Lys Val Leu Gly
  1           5           10          15
Val Trp Ser Ser Pro Phe Val Ile Arg Ala Arg Val Ala Leu Asn Leu
  20          25          30
Lys Gly Leu Ala Tyr Arg Tyr Val Glu Asp Asn Leu Asp Ser Lys Ser
  35          40          45
Glu Leu Leu Leu Ala Ser Asn Pro Val His Gly Lys Val Pro Val Leu
  50          55          60
Leu His Asp Gly Arg Pro Val Cys Glu Ser Arg Val Ile Val Glu Tyr
  65          70          75          80
Ile Asp Glu Ala Phe Pro Ala Ser Gly Pro Cys Leu Leu Pro Ala Asp
  85          90          95
Pro Tyr Arg Arg Ala Val Asp Arg Phe Trp Ala Ser Tyr Ala Asp Asp
 100         105         110
Lys Leu Phe Pro Thr Trp Ile Pro Val Tyr Asn Gly Arg Thr Ser Glu
 115         120         125
Asp Arg Val Ala Ala Ala Arg Gln Val Val Ala Val Leu Glu Lys Phe
 130         135         140
Glu Gln Ala Phe Asp Glu Cys Ser Gly Ser Gly Gly Lys Ala Phe Phe
 145         150         155         160
Gly Gly Asp Ala Ala Gly Leu Val Asp Val Val Leu Gly Gly Phe Leu
 165         170         175
Gly Trp Leu Arg Ala Ser Glu Ala Met Cys Gly Val Arg Val Ile Asp
 180         185         190
Pro Ala Lys Thr Pro Leu Leu Ala Ala Trp Ala Asp Arg Phe Ala Ala

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195	200	205	
Leu Asp Gly Val Arg Glu Val Ile Pro Asp Val Gln Arg Leu Leu Glu			
210	215	220	
Tyr Asn Lys Ile Arg Arg Ala Arg Arg Gly Leu Pro			
225	230	235	

<210> SEQ ID NO 69  
 <211> LENGTH: 904  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 69

```

gacaccacag gcataacaga acagaacat cgatcgagct tggttgctcg gcagcagcta 60
gcaatggccg ccaatggagg tgatgagctg aagctgctgg gcgtgtggga cagcccgtac 120
gtcaacaggg tccagatcgt gctcaacctc aagggcctca gctacgagta cgtggaggag 180
gacctcctca gcaagagcga gtcctcctc aattccaacc cggcgcacaa gaaagtgcc 240
gtgctcatcc acgccgcaa gccggtcgcc gagtcgcagg ccatcgttca gtacctcgac 300
gaggctttcc ccagcggcac gttcccgtcg gtctcccag ccgaacccta cgcacgcgcc 360
accgcccgct tctgggccc cttcgtcgac gacaaggctg ggtctccatg gcacacggtc 420
ctgttcgccc gggagcacgg gaagaaggcg gacgcggcgt cgcggatcgt cgcggcgtg 480
gagacgctgg aggggtgctt cgaggactgc tccggcggga gggactactt cggcggcgac 540
gccatcggct tcgtggacgt ggtcctcggc agctacctgg gctggttcaa ggtgttcgag 600
aagatggctg gcgtcagggt cctggacgtg gcgaggacgc cgctcctcgc cgcgtggggg 660
gagcgtttcg cggcggcgga agcggccaag gacgtcctgc cggatgacgt tgacaaggtg 720
ctcgagttcc ttcagaagtt cctggattag atgcgcgcca ccatgtgctc cgggtgtccaa 780
ctcccaatgt ttgtttgctt tggtcatttt cgggtgcgtg ttaatgggccc tcggatgttt 840
gccagttgat taacttgatt ttatagaatc ttaataatat tctaaaacaa aaaaaaaaaa 900
aaaa 904

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<210> SEQ ID NO 70  
 <211> LENGTH: 228  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 70

Met Ala Ala Asn Gly Gly Asp Glu Leu Lys Leu Leu Gly Val Trp Asp	
1 5 10 15	
Ser Pro Tyr Val Asn Arg Val Gln Ile Val Leu Asn Leu Lys Gly Leu	
20 25 30	
Ser Tyr Glu Tyr Val Glu Glu Asp Leu Leu Ser Lys Ser Glu Leu Leu	
35 40 45	
Leu Asn Ser Asn Pro Val His Lys Lys Val Pro Val Leu Ile His Ala	
50 55 60	
Gly Lys Pro Val Ala Glu Ser Gln Ala Ile Val Gln Tyr Leu Asp Glu	
65 70 75 80	
Ala Phe Pro Ser Gly Thr Phe Pro Ser Val Leu Pro Ala Glu Pro Tyr	
85 90 95	
Ala Arg Ala Thr Ala Arg Phe Trp Ala Ala Phe Val Asp Asp Lys Val	
100 105 110	
Gly Ser Pro Trp His Thr Val Leu Phe Ala Arg Glu His Gly Lys Lys	
115 120 125	



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Ala Asp Ala Ala Ser Arg Ile Val Ala Ala Leu Glu Thr Leu Glu Gly  
 130 135 140

Ala Phe Glu Asp Cys Ser Gly Gly Arg Asp Tyr Phe Gly Gly Asp Ala  
 145 150 155 160

Ile Gly Phe Val Asp Val Val Leu Gly Ser Tyr Leu Gly Trp Phe Lys  
 165 170 175

Val Phe Glu Lys Met Val Gly Val Arg Val Leu Asp Val Ala Arg Thr  
 180 185 190

Pro Leu Leu Ala Ala Trp Gly Glu Arg Phe Ala Ala Ala Glu Ala Ala  
 195 200 205

Lys Asp Val Leu Pro Asp Asp Val Asp Lys Val Leu Glu Phe Leu Gln  
 210 215 220

Lys Phe Leu Asp  
 225

<210> SEQ ID NO 71  
 <211> LENGTH: 1013  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 71

ctggtctctt gcacagactc ggagcaagat aggcctagct ggttacaagc caagaacaag 60  
 tagagcggta gagggaaat atatctggag agggaaacaa tggcgggcca ggagggtctt 120  
 aaggtcctcg gcctgcaggt gagccggtc gtgctccgcg tgtgcttggc gctgaacatg 180  
 aaaggagtga gttacgagta cgtcgaggag gacatatcca acaagagtga gctcctgctc 240  
 aagtccaacc cggtgcaaaa gaaggtgccc gtgctcatcc acaacggtaa gcccatctgc 300  
 gagtcactcg tcatcatgca gtacgtcgac gagctgttcg ccggccggcc gatcctccca 360  
 accgaccct acgagcgcgc cactgctcgc ttctgggctg cctacgccga cgacaagttg 420  
 tttccagcgt ggtacggcat ggtgaaggcc cagccggagg aggagagggc ggagaaggcg 480  
 aaggagacgc tcgcccat cgagcacatg gaagtgcct tcgccaagtg ctccggcggc 540  
 aacgccttct tcggtggcga ctccatcggc tacgtcgaca tcgtgctgac gtgctcggct 600  
 ccttcttggt ctggttcgag gcggtgcgca gggttttcga cctggagatc attaacgcta 660  
 gcaagactcc gctggtggct gcgtgggccc agcggtttgt agggactgta gaagcgaagg 720  
 aggtggtgcc gttgccacg gcggacatgg cggtacagta catcaataag cttcatgccc 780  
 ccctgccgcc gccatgagtt cacaatgagt cgtgtaagt taaccaagca gggaaaaagg 840  
 taaatggtgc ggtgctttg tccaaattcc aaccgaataa tgttcaaagc ttatattgat 900  
 aggcttgggt tgttgcctc aaatattgtg gttcagtcgt ctcctctgca ataaatataa 960  
 atatgattat tactttcttt gccgtaaaaa aaaaaaaaaa aaaaaaaaaa aaa 1013

<210> SEQ ID NO 72  
 <211> LENGTH: 200  
 <212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 72

Met Ala Gly Glu Glu Gly Leu Lys Val Leu Gly Leu Gln Val Ser Pro  
 1 5 10 15

Phe Val Leu Arg Val Cys Leu Ala Leu Asn Met Lys Gly Val Ser Tyr  
 20 25 30

Glu Tyr Val Glu Glu Asp Ile Ser Asn Lys Ser Glu Leu Leu Leu Lys

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35					40					45					
Ser	Asn	Pro	Val	His	Lys	Lys	Val	Pro	Val	Leu	Ile	His	Asn	Gly	Lys
50					55					60					
Pro	Ile	Cys	Glu	Ser	Leu	Val	Ile	Met	Gln	Tyr	Val	Asp	Glu	Leu	Phe
65					70					75					80
Ala	Gly	Arg	Pro	Ile	Leu	Pro	Thr	Asp	Pro	Tyr	Glu	Arg	Ala	Thr	Ala
				85					90					95	
Arg	Xaa	Trp	Ala	Ala	Tyr	Ala	Asp	Asp	Lys	Leu	Phe	Pro	Ala	Trp	Tyr
			100					105					110		
Gly	Met	Val	Lys	Ala	Gln	Pro	Glu	Glu	Glu	Arg	Ala	Glu	Lys	Ala	Lys
		115					120					125			
Glu	Thr	Leu	Ala	Ala	Ile	Glu	His	Met	Glu	Val	Thr	Phe	Ala	Lys	Cys
		130					135					140			
Ser	Gly	Gly	Asn	Ala	Phe	Phe	Gly	Gly	Asp	Ser	Ile	Gly	Tyr	Val	Asp
145					150					155					160
Ile	Val	Leu	Thr	Cys	Ser	Ala	Pro	Ser	Cys	Ser	Gly	Ser	Arg	Arg	Cys
				165					170					175	
Ala	Gly	Phe	Ser	Thr	Trp	Arg	Ser	Leu	Thr	Leu	Ala	Arg	Leu	Arg	Cys
			180					185					190		
Trp	Leu	Arg	Gly	Arg	Ser	Gly	Leu								
		195					200								

<210> SEQ ID NO 73  
 <211> LENGTH: 1068  
 <212> TYPE: DNA  
 <213> ORGANISM: maize

<400> SEQUENCE: 73

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ggcagcacia acgagcacia taatggccgg aggagggtgac gatgaactca agctgctggg      60
ggcgtggggcg agccattcgc tctgctgggt gaagctcgcg ctgagcttca agggcctgag      120
ctacgaggac gtggaggagg acctctccgg cggcaagagc gagctgctcc tgcagtccaa      180
cccgggtcac aagaaggtgc ccgtgctcct ccacaacggc aagcctgtgt gcgagtcgca      240
gatcatcgtg cagtacatcg atgaggcctt cgccggcact ggcccgtccc ttctccctgc      300
cgacccgcac cagcgcgccg tggctcgctt ctgggggtgcc tacattgacg acaagctcct      360
agccttctgg ctgcaatcag caagggccaa gacgcaggag gaaaaggccg aggcgctgaa      420
gcaggcgctc gccgcggccg agaacctgga ggccgccttc acggagatct ccgagggcaa      480
gcccttcttc ggcgcgaca gcgtcgggta cctggacgtg acgctgggag cgctggctgc      540
gtgggtgcac gccgccgaga agctgtacgg gatgaggctc ttcgacgcca cgaggacccc      600
gctgctgagc gcgttcgtgg agaggttcgg cgcgctcgga gcggccaagg cgggtgctgc      660
cgacgtcgat gccctcgtcg aatacgccaa acagaggcag gccgacgagg cagctgcagc      720
ctcggacagc taaaaaatg gcaccgcgag tttaccgacg tacggcagtc agtgctggac      780
gaagcaagat tatgggtatt ctgcatatac tattcagctg ctgtcgtgtg tattagctgg      840
ttgttactag attgttggcg tgtgacaaaag aaaataaaaa tggatggggc ggctttcgtt      900
tgtgtttgta ttgtacgttt gccgtttggt gtgtaccgtg tgtcgtaggt cggaaattgc      960
cgcatatcgg catgcctagt gtaaccctgt cgattatgca gtctggtttg ctttatattc     1020
accaaagtaa gtaatctgaa taattttctt gaaaaaaaa aaaaaaaaaa                    1068

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<210> SEQ ID NO 74  
 <211> LENGTH: 236



-continued

<212> TYPE: PRT  
 <213> ORGANISM: maize

<400> SEQUENCE: 74

Met Ala Gly Gly Gly Asp Asp Glu Leu Lys Leu Leu Gly Ala Trp Ala  
 1 5 10 15  
 Ser Pro Phe Val Leu Arg Val Lys Leu Ala Leu Ser Phe Lys Gly Leu  
 20 25 30  
 Ser Tyr Glu Asp Val Glu Glu Asp Leu Ser Gly Gly Lys Ser Glu Leu  
 35 40 45  
 Leu Leu Glu Ser Asn Pro Val His Lys Lys Val Pro Val Leu Leu His  
 50 55 60  
 Asn Gly Lys Pro Val Cys Glu Ser Gln Ile Ile Val Gln Tyr Ile Asp  
 65 70 75 80  
 Glu Ala Phe Ala Gly Thr Gly Pro Ser Leu Leu Pro Ala Asp Pro His  
 85 90 95  
 Gln Arg Ala Val Ala Arg Phe Trp Gly Ala Tyr Ile Asp Asp Lys Leu  
 100 105 110  
 Leu Ala Phe Trp Leu Gln Ser Ala Arg Ala Lys Thr Gln Glu Glu Lys  
 115 120 125  
 Ala Glu Ala Leu Lys Gln Ala Leu Ala Ala Ala Glu Asn Leu Glu Ala  
 130 135 140  
 Ala Phe Thr Glu Ile Ser Glu Gly Lys Pro Phe Phe Gly Gly Asp Ser  
 145 150 155 160  
 Val Gly Tyr Leu Asp Val Thr Leu Gly Ala Leu Val Ala Trp Val His  
 165 170 175  
 Ala Ala Glu Lys Leu Tyr Gly Met Arg Leu Phe Asp Ala Thr Arg Thr  
 180 185 190  
 Pro Arg Leu Ser Ala Phe Val Glu Arg Phe Gly Ala Leu Gly Ala Ala  
 195 200 205  
 Lys Ala Val Leu Pro Asp Val Asp Gly Leu Val Glu Tyr Ala Lys Gln  
 210 215 220  
 Arg Gln Ala Asp Ala Ala Ala Ala Ser Asp Ser  
 225 230 235

What is claimed is:

1. An isolated nucleic acid fragment encoding a GST enzyme selected from the group consisting of:

(a) an isolated nucleic acid fragment encoding the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72 and SEQ ID NO:74; and

(b) an isolated nucleic acid fragment that is complementary to (a).

2. The isolated nucleic acid fragment of claim 1 selected from the group consisting of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61,

45 SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71 and SEQ ID NO:73.

3. A chimeric gene comprising the isolated nucleic acid fragment of claim 1 operably linked to suitable regulatory sequences.

40 4. A transformed host cell comprising the chimeric gene of claim 3.

5. The transformed host cell of claim 4 wherein the host cell is a plant cell.

55 6. The transformed host cell of claim 4 wherein the host cell is *E. coli*.

7. A method of altering the level of expression of a GST enzyme in a host cell comprising:

(a) transforming a host cell with the chimeric gene of claim 3 and;

60 (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of altered levels of a GST enzyme in the transformed host cell relative to expression levels of an untransformed host cell.

65 8. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a GST enzyme comprising:

**119**

(a) probing a cDNA or genomic library with a nucleic acid fragment selected from the group consisting of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71 and SEQ ID NO:73, under the following hybridization conditions: 0.1×SSC, 0.1% SDS at 65 degrees C.;

**120**

- (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of step (a); and
- (c) sequencing the cDNA or genomic fragment that comprises the clone identified in step (b),

wherein the sequenced cDNA or genomic fragment encodes all or substantially all of the amino acid sequence encoding a GST enzyme.

\* \* \* \* \*